

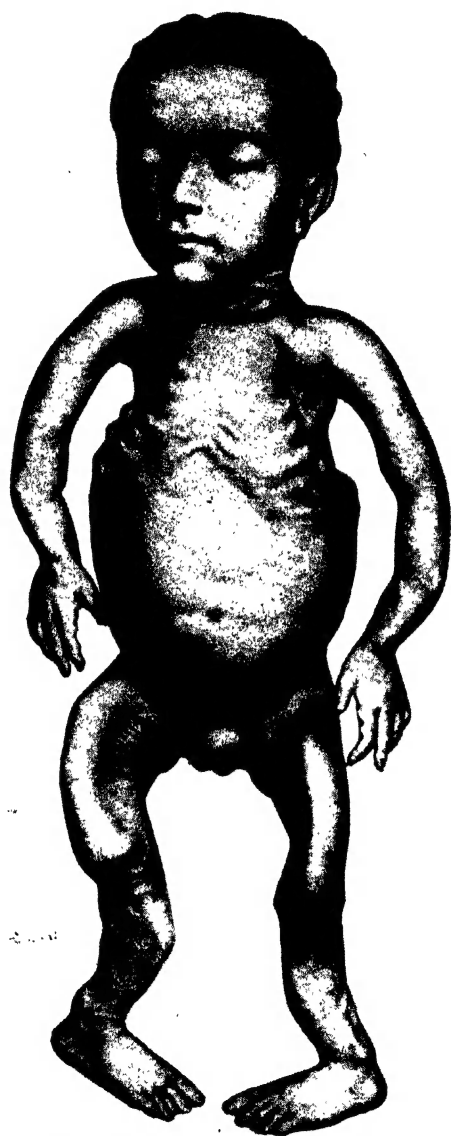
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THE VITAMINS IN MEDICINE

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Enfant Rachitique de 8 ans. Paris 1852.

THE VITAMINS IN MEDICINE

by

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and

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PREFACE TO THE SECOND EDITION

Since the first edition of this book appeared further advances in our knowledge of the vitamins have been made, especially concerning the vitamin B complex and riboflavin, the chapters on which have been largely rewritten and expanded. The other chapters have required less drastic alterations to bring them up to date. A short chapter has been added on the essential unsaturated fatty acids and minor fat-soluble vitamins. The tables giving the vitamin content of foods have also been revised. References to original papers now number nearly 4,500 and the number of illustrations has been increased from 120 to 208.

We are grateful to those who have offered helpful suggestions and criticisms, and to those who have kindly given permission to reproduce original illustrations. We have been asked for an author index but we regretfully decided that the advantages of this were outweighed by the increase this would mean in the size of the book—such an index would contain some twelve thousand names and some thirty thousand page references.

It is a pleasure again to acknowledge the sympathetic assistance given to us by Messrs. William Heinemann, Dr. J. J. Abraham and Mr. L. B. Cavender. Mr. G. F. Home, Librarian of the Royal Society of Medicine, and his staff have also given us most valuable help.

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THE VITAMINS IN MEDICINE

INTRODUCTION

At the beginning of the present century nutritional research was still chiefly concerned with the study of carbohydrates, fats, and proteins. These in their correct proportions, with the addition of minerals such as iron and calcium, were believed to supply all the needs of the body. Indeed at this time the chief problem in human nutrition appeared to be how to provide more animal protein and fat for the poor.

This simple conception of nutrition was accepted until just before the last war, when it was shown that natural unrefined foods contained hitherto unrecognized substances which are essential for life. That the existence of these substances was recognized so late in the history of dietetic research was due to their being present in foods in very minute amounts. To emphasize their vital importance they were named vitamins.

The discovery of vitamins revolutionized the outlook not only in dietetics but also in the study of disease. The realization that many human diseases were not caused by infections or toxins but simply by vitamin deficiencies in the food led to the conception of deficiency diseases.

Vitamins were discovered at a singularly opportune moment. By the beginning of this century there was a great decrease in the consumption of fresh and unrefined foods. The population was migrating from the country to the towns. This led to fresh country produce, such as vegetables milk and eggs, becoming too costly for the urban poor, while their increasing consumption of white flour also seriously impoverished their diet. These insidious changes in the nation's food caused, and still cause, mild or even severe vitamin deficiencies in many of the poor and ignorant.

During the last thirty years an ever-increasing amount of research has been devoted to the study of vitamins. Much of this work has been concerned with fundamental problems dealing with their biological significance, their chemical structure and their methods of estimation. Until such problems had been thoroughly investigated the study of vitamins in relation to dietetics and the treatment of disease could not be put on a sound basis. Now, however, enough is known for medicine to utilize the knowledge that has been gained.

Unfortunately this knowledge is spread throughout the literature of three decades and much of it is not easily available. Its volume is so vast that it disconcerts even those who lay claim to be specialists in the field. Just before the present war over three thousand original papers dealing with the vitamins appeared annually throughout the world. The magnitude of this literature makes it wellnigh impossible for clinicians and others to keep abreast of the subject. Vitaminology like endocrinology affords a common meeting ground for the chemist, biochemist, physiologist, clinician and even the physicist, who has pressed radio-

activity and fluorescence microscopy into service to study the vitamins. We have made an attempt to correlate up-to-date information on the chemistry, physiology, nutritional importance and clinical uses of the vitamins, although we cannot hope to have done full justice to all the publications of recent years, or to have avoided errors of judgment in the selection and interpretation of the available literature. As far as we are aware such information has not previously been collected in a single work. We would, however, like to acknowledge the series of articles written during 1988 and collected in book form in 1989 by the American Medical Association. Numerous advances have been made since this work was published, particularly in our knowledge on vitamins E, K, P, and the vitamin B complex, the picture of which constantly changes, and also in the therapeutic uses of the vitamins.

Until a few years ago the vitamins were only of clinical interest in the treatment of certain deficiency diseases. It is now realized that in large doses they can be used therapeutically in other conditions. Like the hormones vitamins can produce definite pharmacological effects, which can be used therapeutically in subjects who are not suffering from a vitamin deficiency. Nicotinic acid, for example, has a powerful vasodilator action even in a normal person, a property which suggests its use in peripheral vascular disease. Both vitamin B₁ and C stimulate metabolism, and the latter increases the oxygen uptake of blood with a low oxygen tension, indicating that it might be useful in cases of accidents and injuries involving hæmorrhage.

In this book emphasis has been laid upon the clinical aspect of the subject. It is true that clinically vitamins have been used indiscriminately by a number of workers during the last few years, and Walshe decries the tendency of the clinician to mount what he calls the vitamin band-wagon before preliminary work has been critically reviewed. It is useless to expect too much of vitamin therapy. Even its most ardent advocates, for instance, do not pretend that vitamin B₁ is a specific for non-nutritional neuritis; that nicotinic acid will cure deafness; or that vitamin E is a specific for all forms of abortion. But we know that these vitamins have certain physiological or pharmacological effects that can be used to improve the general condition of the patient. Much vitamin therapy is not specific but ancillary to other forms of treatment. Thus the beneficial effect that vitamin B₁ is reputed to have in relieving the pain of certain cases of neuritis may well be due to an effect on the metabolic processes in muscle and nerve tissue; indeed a humoral effect mediating through acetylcholine and an improved blood supply cannot be excluded. Similarly, nicotinic acid has been shown to cause dilatation of the blood vessels in the brain and spinal cord of the experimental animal. This would explain the temporary improvement noted in patients with disseminated sclerosis and high tone deafness when treated with nicotinic acid. Again, healing in tuberculosis is characterized by the formation of connective tissue, for which vitamin C is essential, and it is therefore not unreasonable to suppose that the administration of large doses of the vitamin might aid the healing of tuberculous lesions in the lung.

Clinical experience with vitamins would be greatly advanced if doctors were fully acquainted with work that has already been done, and with the physiological or pharmacological reason underlying the use of a vitamin in any particular condition. Clinical vitamin research offers an ideal field to the general practitioner, who has the opportunity for long and intimate observation. Many of his observations remain unpublished because he does not know whether they are new or old. This is to be regretted, since many common problems in general practice are never seen in hospital work and must be dealt with by the practitioner and not the consultant.

The importance of vitamins in nutrition should not need any emphasis. The problems raised by the vitamin deficiencies in our food are so important that they are incapable of solution by the medical profession alone. Many are social problems requiring the help of the economist and legislator. Wise feeding is the greatest weapon preventive medicine can wield. For years it has been known that the children of London and our big cities have been living on diets so deficient in vitamins that three-quarters have at some time in their lives had rickets and one-half defective twilight vision. The extensive nutritional surveys of Sir John Orr in this country and Stiebeling and Phipard in America have shown that a considerable proportion of the population—Orr puts the figure at 50 per cent.—are receiving insufficient vitamins, particularly those of the B complex, since they live largely on white bread, which far from being the staff of life, is only a broken reed. Civilised man does not instinctively choose a sound diet. A survey of the diets of more than 1,000 workers in a large aircraft factory revealed that more than four-fifths consumed diets considered to be inadequate according to the standards laid down by the Nutritional Board of the National Research Council. Nutritionists who watched workers select their luncheons in cafeterias reported that not more than half chose good lunches even when these were available on the counter. Another survey of nearly 6,000 school children in America revealed that only 11 per cent. of the white and 8 per cent. of the coloured children received a diet adequate in all the essential food factors. It is reported that 40 per cent. of men called up for the American Forces are rejected as being unfit for military service, and Jolliffe states that one-third of these are suffering from disabilities directly or indirectly connected with nutrition.

The majority of the public are apathetic towards the problem and are content to spend their lives in that shadowed region between good health and frank illness. There is a wide gap between the minimum diet, which just prevents deficiency symptoms, and the optimum to promote good health. Subnormal nutrition may be present even when there are no obvious clinical signs. Human subjects who have volunteered to go on experimental diets deficient in one or other of the vitamins generally complain of tiredness before any other recognisable sign of deficiency occurs. This "tiredness" is one of the commonest complaints in the doctor's surgery and it is not unreasonable to associate it in some cases with vitamin deficiency in the patient.

The rise in tuberculosis among adults who are moved from slums into good building estates where rents are higher, so that there is less money available for food, is a painful proof that food and fitness go together. Poor children give an even clearer picture of the effects of malnutrition, being smaller than their richer brothers and sisters, more prone to infection, and with a higher death rate. It has been repeatedly demonstrated that school children show considerable improvement in weight, height, physique, and the condition of their skin and hair when given dietary supplements rich in vitamins such as milk or the Oslo breakfast. Malnutrition is an important factor in infant mortality, the rate of which is twice as high in poor districts as in wealthy ones. Some returns from Newcastle-on-Tyne show that the measles mortality rate was three times as high in the poor districts and the incidence of bronchitis and pneumonia as complications eight times as high. The poor quality rather than the quantity of the food is certainly one of the factors responsible. How the problem of malnutrition will be solved we cannot say. It is an economic and educational problem as well as one of medicine and public health.

For the sake of simplicity deficiency diseases are discussed under separate vitamins, *e.g.*, beriberi under vitamin B₁, pellagra under nicotinic acid, and scurvy under vitamin C. This may give the impression that such diseases are due to lack of a single vitamin. This is not so. With the exception of rickets, deficiency diseases are due to multiple deficiencies and not to deficiency of a single factor. Even a professor of dietetics would find it difficult to devise a diet lacking in just one factor. We do not know the precise rôle played by lack of protein, essential amino-acids and minerals in deficiency diseases such as beriberi and pellagra. Nor do we know of the inter-relationships between the various vitamins and other essential food factors. Will a unit amount of a vitamin give the same response when fed as a free vitamin with a synthetic diet as when it is supplied in natural foodstuff containing other factors? There is clinical evidence that the vitamin B complex is better than vitamin B₁ alone, and in the animal it has been shown that there is better growth with vitamin B₁ when it is given with liver, *p*-aminobenzoic acid and inositol than when it is given alone.

We have hesitated to give a definition of a vitamin, which in the present state of our knowledge is beset with difficulty. What is a vitamin for one species is not a vitamin for another. Thus the rat has no need of vitamin C, which it can synthesise, yet this is essential for man and many other animals. Broadly speaking, a vitamin is an organic compound essential for the maintenance of the life process; it cannot be synthesised in adequate quantities by the organism requiring it; it is only required in small amounts; it does not furnish energy by its metabolism, nor do its molecules contribute to tissue building or storage elements of the organism; it is a catalyst essential either for the transformation of energy, for the working of certain enzyme systems, or for the regulation of the metabolism of certain tissues. Admittedly this definition does not cover all the vitamins. It is true that some of the B vitamins are concerned with energy transformation, vitamins A and C with the metabolism of epithelial

tissue and connective tissue respectively, and vitamin D with phosphorus metabolism throughout the body. But this definition does not explain the nature of vitamin K—unless this is a component of an oxidation—reduction or enzyme system (p. 806)—or the essential fatty acids, or vitamin E, which probably functions as an anti-oxidant. It is still debatable whether such substances as inositol and choline, which are present in comparatively large amounts in the diet, should be classed as vitamins. Is methionine a vitamin?

The major vitamins are considered in this work under the following headings: history, chemistry, food sources, physiology, pharmacology, human requirements in health and disease, deficiency diseases, methods for detecting vitamin deficiencies and therapeutic uses.

CHAPTER I

VITAMIN A

THE ANTIXEROPHTHALMIC VITAMIN

THE ANTI-INFECTIVE VITAMIN

AXEROPHTOL

VITAMIN A is the term used to include vitamin A₁ found in animals and sea fish, and vitamin A₂ found chiefly in fresh-water fish. Biologically and chemically they are so very similar that generally no distinction is made between them. The little that is known about vitamin A₂ is discussed on p. 81. Axerophthol was the name given by Karrer in 1938 to vitamin A₁, but it has not come into common use.

Provitamin A is the name given to those plant carotenoids which can be converted by animals to vitamin A.

HISTORY

The most clear-cut effect of lack of vitamin A is night blindness, which often occurs suddenly after long exposure to a day's bright sunlight. In rural communities inability to see in the dusk is a very serious condition: fishermen, for instance, may walk off the rocks into the sea after landing in the evening. Night blindness can be cured, often in twelve hours, by eating food rich in vitamin A, such as liver. The dramatic quickness both of the onset and the cure explains why liver has been used for centuries for night blindness.

The Ebers Papyrus [1], written about 1600 B.C., probably referred to night blindness when liver was recommended for the eyes, while the Chinese in 1500 B.C. were giving liver, honey, flying fox dung and tortoise-shell, all of which would have cured night blindness [5]. Hippocrates advised the whole liver of an ox dipped in honey, and liver was known to later Roman writers. Jacob van Maerland, a Dutch poet of the fourteenth century, may be thus translated [2],

He who cannot see at night,
Must eat the liver of the goat
Then he can see all right.

Guillemeau in France in the sixteenth century besides clearly describing night blindness, advised liver for its cure [8], which was also advised by other writers at that time [5].

Drummond and Wilbraham [2] find that the first mention of liver for the eyes in England was in Muffett's "Health's Improvement" (1655), though Bayly, at one time Queen Elizabeth's physician, in his book on eyes recommends "rawe herbes" among which is "eie bright"; but the only evidence of night blindness being common at this time is references to mists and films over the eyes. "Rawe herbes" would of course provide provitamin A.

Aykroyd [1] in his accounts of Newfoundland and Labrador fishermen says they not only recognize how bright sunlight may bring on night blindness, but also use liver, preferably the raw liver of a gull or puffin, for a cure.

The beginning of the present century saw the realization that more serious eye affections—especially “conjunctivitis” in children—were due to lack of some food factor. Mori [4] in Japan in 1904 treated juvenile conjunctivitis with cod-liver oil, believing the diet was inadequate in fats, while Monrad in 1917 thought that the outbreak of conjunctivitis which occurred in Danish children at that time was due to a deficiency of a fat soluble factor, caused by the export of the country’s animal fats to England and Germany.

Animal experiments as early as 1909 had shown that rats on deficient diets developed conjunctivitis [6]; that a fat soluble factor was involved was proved by Osborne and Mendel [7] in 1918, and McCallum and Simmonds [8] in 1917. The latter workers called the factor “fat soluble A” and pointed out the similarity between xerophthalmia in rats and the conjunctivitis found in children on fat-deficient diets.

Conjunctivitis and xerophthalmia are, however, only the most noticeable examples of the change in the epithelial surfaces of the body brought about by lack of vitamin A. Wolbach and Howe [9] in 1925 found that “the specific tissue change due to deprivation of fat soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium.” This replacement of a specialized epithelium by a primitive type leads to a lowered local resistance to infection.

The name “anti-infective” vitamin was first given to vitamin A by Green and Mellanby [10] in 1928 because they found that animals killed by lack of vitamin A showed multiple foci of infection in those areas where the epithelium had altered. At this time the infection was regarded as the direct, and not secondary, effect of lack of vitamin A.

The separation of vitamin D from vitamin A was not complete before 1925. As early as 1909 Stepp [11] had found that there was some unrecognized factor in fats necessary for growth, and in 1918 McCollum and Davis [12] and also Osborne and Mendel [7] confirmed this, the latter workers also stressing that different fats varied in their value for growth. Mellanby in his work on rickets from 1918 onwards originally believed that the antirachitic factor, whose existence he discovered, was the same as the fat soluble A factor of McCollum and Davis. But in 1922 and the following years several very important papers appeared, all showing that there were two separate factors in fats—the growth promoting or anti-xerophthalmic factor and the antirachitic factor. Thus Hume [18] and also Goldblatt and Soames [14] found that ultra-violet irradiation, while it cured rickets, would not prevent xerophthalmia or maintain growth in animals on fat deficient diets. A year later in 1928 Goldblatt and Zilva [15] found that the growth-promoting and antirachitic functions of cod-liver oil were destroyed by heat and oxidation at different rates, and they also observed that spinach was excellent for growth but not for preventing rickets. Mellanby [16] in 1926, comparing the diets of a series of puppies

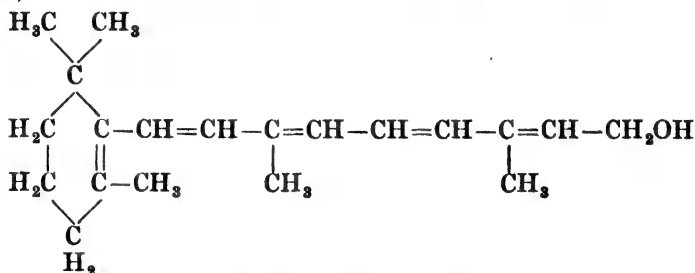
who had died or survived an epidemic of bronchopneumonia, reported that the protective value of the diet against infection was not related to its protective value against rickets.

The carotene content of plants was found by Rosenheim and Drummond [17] in 1920 and by Coward [20] in 1923 to vary with their vitamin A potency, a relationship which was further emphasized by Rosenheim and Drummond's [18] observations in 1925 on the similarity of the colour reactions of the two. Between 1929 and 1930 Moore [21, 22, 23] and Capper [24] were largely responsible for showing that carotene could be used by animals as a source of vitamin A, into which it is converted in the body.

The chemistry, isolation and synthesis of vitamin A and its relationship to carotene was settled chiefly by the work of Karrer [25, 26, 27] and Heilbron [28, 29] and their co-workers and of Holmes and Corbett [30] between 1930 and 1937.

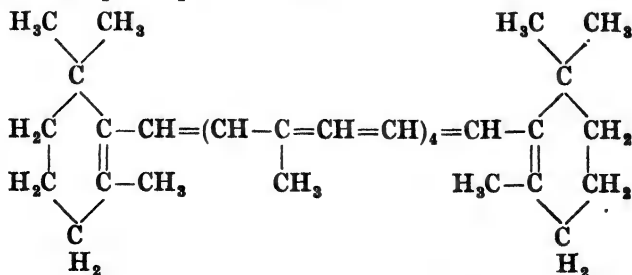
CHEMISTRY OF VITAMIN A

Karrer and his collaborators [25, 26, 27, 34, 35] first suggested the now accepted formula for vitamin A, which was confirmed by Heilbron and others [28, 36] in England, and also by Euler [37]. Vitamin A has the formula,



being formed in the body from one of its carotenoid provitamins, alpha-, beta-, and gamma-carotene and cryptoxanthine [31], and a few other rare carotenoids (p. 11).

Beta-carotene [25, 34] has the formula



If the β -carotene molecule is split in two at the middle of the central aliphatic chain by hydrolysis two identical alcohols are formed, each being a molecule of vitamin A. The conversion of β -carotene to vitamin A has

not been achieved chemically, though it is known to occur in living animals, possibly through the action of the liver enzyme carotenase (p. 18). It appears probable that in the body the β -carotene molecule is not split into two symmetrical halves, with the result that only half the maximum possible amount of vitamin A is formed (p. 18).

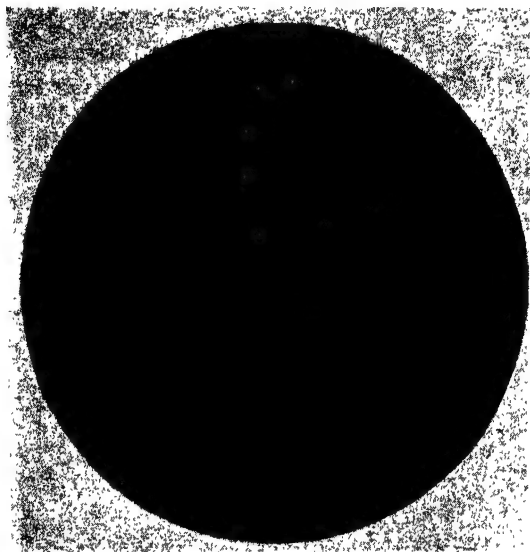
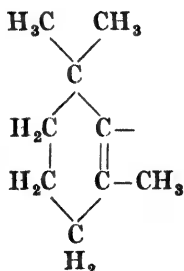


FIG. 1. Crystals of Carotene (Provitamin A).

The other three common provitamins only have one β -ionone ring, so that on hydrolysis each molecule can at most form only one molecule of vitamin A. For biological activity the presence of the unsubstituted β -ionone nucleus



is necessary [85, 332].

The synthesis of vitamin A was achieved in 1937 by Kuhn and Morris [38], though it was not obtained in a pure form. Holmes and Corbett [30] in the same year isolated from fish liver oil a crystalline vitamin A preparation which appeared to be the pure vitamin. The crystals were pale yellow, melting at $5.5^\circ\text{--}6^\circ\text{C}$. to a viscous oil. The blue value (p. 10)

was 100,000 and $E \frac{1\%}{1 \text{ cm.}}$ 828 equalled 2,000. The biological activity was reported to be 3,000,000 I.U. per gram [57], which gave a conversion factor nearer 1,500 than 1,600 (p. 8). Mead, Underhill, and Coward [68] in 1939, using two crystalline esters of vitamin A, reported that the biological activity was 3,181,000 and 3,424,000 I.U. per gram, and since $E \frac{1\%}{1 \text{ cm.}}$ 828 equalled 1,600–1,800, the conversion factor was about 2,000.

The yellow crystals of the vitamin A alcohol made by Baxter and Robinson [30] in 1940 melted at 63°–64° C. and had a biological activity of 2,700,000 I.U. per gram, with $E \frac{1\%}{1 \text{ cm.}}$ 828 equalling 1,725 and $E \frac{1\%}{1 \text{ cm.}}$

622 equalling 4,700. A later paper [385] by the same workers should be read in full: details are given about four vitamin A esters and nine preparations of vitamin A alcohol. The high conversion factors and biological activity which are reported for the esters and alcohol are probably due to the use of the Reference Cod Liver Oil of the United States Pharmacopœia (pp. 9, 10).

Vitamin A gives a band of maximum absorption in the ultra-violet region of 828 millimicrons [50, 51].

Vitamin A exhibits fluorescence when irradiated by a mercury vapour lamp [383]: this has been used by Popper [290] to demonstrate vitamin A in the tissues (Figs. 2, 11, 12) by the technique which he describes in the *Archives of Pathology* [290]. The differences in the fluorescence of the alcohol and the ester have been used to determine the amounts of each in preparations of vitamin A [383].

Biological activity is lost when vitamin A takes part in chemical reactions which affect double bonds, such as oxidation, hydrogenation and bromination [39]. Heat does not destroy vitamin A in butter at temperatures below about 120° C. in the absence of oxygen [40]; when oxygen is present slow destruction occurs in oils even at room temperatures [41] and rancidity accelerates this, though it probably causes no appreciable loss in human food [278]. Many fats when heated develop an "anti-vitamin A" factor which destroys the biological activity of vitamin A: whether this is a chemical or biological effect is unknown [319, 320]. Exposure of cod liver oil to light reduces its vitamin A content, so the oil should be stored in dark bottles [42, 43]. Vitamin A in "cod liver oils" prepared from concentrated fish liver oils diluted with cottonseed, peanut or maize oil is less stable than vitamin A in genuine cod liver oil, nearly twice as much being destroyed after exposure to winter sunshine for eleven days [334]. (See also p 45.)

Vitamin A and its carotenoid precursors being fat soluble and unsaponifiable are found concentrated in the unsaponifiable extracts of fats. Separation of vitamin A from the carotenoids can be carried out by dissolving both in petroleum ether and adding alcohol, when the latter will dissolve the vitamin A but not the carotenoids. Since alcohol and petroleum ether are not miscible, the alcohol layer containing the vitamin

can be easily separated. Vitamin A esters, however, remain with the carotenoids.

The colour reactions of vitamin A are of great practical importance. They occur with sulphuric acid [17] and with the chlorides of polyvalent metals [18, 19]; the most satisfactory reagent was found by Carr and Price [19] to be antimony trichloride dissolved in chloroform. This test is often called the Carr-Price colour test. With vitamin A a blue colour develops rapidly and then fades. The blue colour, however, is not only given by vitamin A but also by carotenoids [18] and "oxycholesterol" [44]. Therefore to confirm the presence of vitamin A the absorption spectrum of the Carr-Price reaction must be examined, when, if vitamin A is present, specific absorption bands will be found. To demonstrate the presence of vitamin A within the tissues of the body Popper [290] uses fluorescence microscopy.

Liver oils are the substances most often examined by the Carr-Price reaction. Two bands of maximum absorption are found at 572 and 606 millimicrons, which in concentrated solutions are displaced to 583 and 620 [45]. The band at 572 or 583 appears to be due to vitamin A, since it varies in intensity both with the biological value of the oil, and also with the ultra-violet absorption band at 328, which is due to vitamin A itself [45, 47]. The band at 606 or 620 varies in intensity with the age of the oil, apparently owing to slow oxidation, so that its intensity bears no constant relationship either to that of the band at 527 or that of vitamin A at 828 unless the oil is fresh [46]. It can be removed from the Carr-Price colour reaction by the addition of 7-methylindole, while the band at 572 is not affected [48]. It also may appear more intense than it really is by being overlapped by a band due to vitamin A₂ (p. 81) with a maximum intensity at 645-650 [49].

ESTIMATION OF VITAMIN A AND CAROTENE

Vitamin A can be estimated biologically; physically, by absorption spectra estimations; and chemically, by colour reactions. Since the same methods are used in the estimation of carotene, this will not be described; Morton's book [871] and recent papers [872] should be consulted for accounts of the physico-chemical assay of the carotenoids and vitamin A both when these occur alone and together.

The Biological Estimation. In biological estimations—which have been fully discussed by Coward [52] in her book on the subject—either the curative or the prophylactic method can be used; that is the potency of the substance being tested is estimated either by its capacity to cure the symptoms of vitamin A deficiency or by its capacity to prevent their onset. Of the two methods, the curative is generally considered more satisfactory, though it has been criticized firstly on the grounds that the ill-health of the vitamin A depleted animals at the beginning of the estimation varies, this variation altering their subsequent response to vitamin A, and secondly on the grounds that the "depletion period" during which a vitamin A deficient diet is given is avoided in prophylactic tests. Coward [52] has pointed out that neither criticism is valid because

firstly deficient rats show no greater individual variations than do normal rats in their response to vitamin A, and secondly in the prophylactic test the animals at the beginning have different amounts of vitamin A already stored, thus introducing a variation in response which can only be ignored if the average results from a large number of rats are used. Various symptoms may be taken for indicating the onset of cure of the deficient state. Of these change in weight has been the most widely used, and has the great advantage that it is easy to measure. Against it has been urged that it is not specific for vitamin A—even though vitamin A has a specific action on growth [858]—since any other deficiency has the same effect. Xerophthalmia (p. 24) and the changes in the desquamated epithelial cells of the vagina (p. 28) are sometimes used, being only caused by lack of vitamin A. Deciding, however, when early xerophthalmia is present or when it is cured depends on what criteria each worker adopts, and so is liable to great variation. The changes in the vaginal epithelium are difficult to interpret [56] unless the animals are previously spayed to prevent the changes due to oestrus [69].

Irving and Richards [70] in 1940 found that after seven weeks on a vitamin A deficient diet young rats had a constant degeneration of nervous tracts in the medulla. There was only a very small difference between the amounts of vitamin A which could and could not protect the rats against this degeneration. It seems possible that this method will give more clear-cut results than those at present in use.

Since the most widely used method of biological estimation is the curative based on the growth response this will be discussed fully; the same principles apply to the other methods [52].

The young rats used for estimations have to be first given a diet deficient in vitamin A until they stop growing or start to lose weight. This is called the depletion period; it can be shortened by having given the suckling mothers a diet lacking in vitamin A. At the beginning of the estimation the rats should weigh about 70 to 90 grams, and should not have severe xerophthalmia. Their diet must of course contain all essentials apart from vitamin A so that their lack of growth and its resumption can only be caused by it [71]. Recently the importance of ample vitamin E in the diet has been emphasized (p. 18). When the rats have been depleted of their stores of vitamin A they are divided into two groups, each containing similar proportions of males and females. The most accurate results are obtained by dividing pairs of littermate rats between the two groups [886]. One group is fed the substance under investigation and one the standard preparation of vitamin A. By comparing the growth of the two the vitamin A content of the first can be determined. The method is accurate to within thirty-three per cent. [51]. The period of the test feeding should last four weeks or longer, the vitamin A preparations being given daily or twice weekly [58].

Gain in weight, however, is not directly proportional to the amount of vitamin A given; doubling the vitamin A does not double the growth. Therefore if 1 gram of an oil of unknown potency causes a gain of weight of 8 grams and 1 gram of the standard oil causes a gain of 4 grams, the

unknown oil is not twice as potent as the standard oil, but possibly only about one and a half times as potent. To enable a comparison to be made between substances causing different gains in weight Coward has made a curve of reference (by feeding groups of rats on varying amounts of the same oil) which shows how weight increases in proportion to the intake of vitamin A. By referring the gain of weight on two different oils to this curve, the relative values of the two to each other can be decided [52, 54, 55].

Even in rats from the same colony under the same conditions the rate of growth for the same amount of vitamin A may differ at different times; so the growth response from the standard vitamin A preparation must be worked out afresh for every fresh group of biological estimations. A laboratory cannot once work out the response of its animals to the standard, and use the results for reference in all subsequent work [51]; Coward [52], for instance, found that had she done this, one sample of oil would have appeared to contain five times the amount of vitamin A it did nine months earlier.

Physical Estimation. Absorption spectrum estimations of vitamin A have been found to be reliable, giving results in harmony with biological estimations as long as the unsaponifiable fraction is used when the substance being tested contains less than 10,000 I.U. per gram of vitamin A. The reason for using the unsaponifiable fraction is that in oils there are substances other than vitamin A which increase absorption in the region of 828 millimicrons. These are removed by saponification, while vitamin A is not if the correct method is employed [51], though it has been recently reported that the presence of some particular fish liver oils during saponification causes a loss of thirty per cent. [344].

It is necessary to be able to convert the results of absorption spectrum estimations into International Units. "It has been found that, within certain defined conditions, measurement of the coefficient of absorption (E) at 828 millimicrons affords a reliable method for measuring the vitamin A content of liver oils and concentrates. As a means of con-

verting values obtained for $E \frac{1\%}{1 \text{ cm.}}$ 828 millimicrons into a figure repre-

senting the International Units of vitamin A per gram of the material examined, the factor 1,600 is recommended for adoption" and "the intensity of absorption at 828 millimicrons may be determined to within ± 2.5 per cent. by any of the recognized methods of spectrophotometry" (Report of the Second International Conference, 1934, on Vitamin Standardisation of the League of Nations). Since 1934 the conversion factor has been variously reported as less than 1,600 and about 2,000 (p. 9), and a further complication has been introduced by work suggesting that vitamin A as the naturally occurring ester is more biologically active than when it is converted into the alcohol during the process of concentrating oils by saponification. Thus Emmett and Bird [72] in 1937 found that neither partial nor complete saponification of an oil materially altered the value of the coefficient of absorption, but did greatly alter the biological value, so that the conversion factor was reduced by

saponification from 1970 to 905. In 1939 Grab [73] and other German workers [74] also found that the natural ester was twice as biologically active as the alcohol, obtaining the conversion factors of $1,800 \pm 400$ for the alcohol and $3,400 \pm 600$ for the ester; the remarkable conversion factor for the ester is explained by saying it is in its natural state. But English work shows no difference in the biological activity of the ester and alcohol [332] and this is supported by recent American work, though different esters have slightly different conversion factors [335]. These results need further confirmation, especially as many vitamin A concentrates now on the market are in the form of the alcohol. Cyclization [348] may also introduce an error, since in old oils the vitamin forms a dehydration compound, losing some of its biological activity without a commensurate alteration in its coefficient of absorption [75]. Morton [76] reviewing the subject in 1940 came to the conclusion that there was no reason to change the conversion factor from 1,600, but the vitamin A sub-committee of the Accessory Food Factors Committee of the Medical Research Council [337] reported in 1943 that the conversion factor should be 1,740. It was also suggested that the use in America of a conversion factor of 2,000 was due to the U.S.P. reference cod liver oil (p. 10) being overvalued [337], so that different conversion factors should be used according to which unit, International or American, is being used.

Irwin's most valuable paper [347] analyses statistically biological vitamin A assays, carried out in nine or ten different laboratories, on halibut liver oil, the U.S.P. reference oil and vitamin A naphthoate to determine the conversion factor. The average for the halibut liver oil was 1,570, for the U.S.P. reference oil 1,820, and for the naphthoate 1,770. These values it is concluded, from their logarithms and their standard errors, are consistent with the same conversion factors for all three substances. Pooling these results gives the conversion factor, mentioned above, of 1,740 with limits of error of ± 120 .

Chemical Estimation. Colour reactions (p. 6) for the estimation of vitamin A are chiefly used for oils and concentrates and the blood [331, 372]. The blue colour of the Carr-Price reaction can be compared directly to Lovibond's graded glasses, giving "Lovibond's blue units" or the intensity of the absorption of the Carr-Price reaction at either 606 or 572 millimicrons [47] can be measured (p. 6). The unsaponifiable fraction must be used, though this is less important for fresh oils [47]. Estimations based on colour reactions do not give such accurate results as the biological and physical estimations [47, 51, 52] and there is no conversion factor to enable the results to be expressed as International Units [57, 59].

UNITS OF VITAMIN A

Since pure vitamin A has not yet become available in any quantity it has not been possible to define the International Unit as a definite weight of the vitamin. Instead a definite weight of β -carotene is used for the International Unit, since it is converted in the body to vitamin A (p. 13) and so may be regarded as the vitamin itself. When the first International Unit for vitamin A was adopted in 1931, it was defined as the vitamin A

activity of 1 microgram of a special sample of carotene. By 1934 this sample of carotene was known not to be a single substance, and so in its place was put a sample of what was thought to be pure β -carotene, of which 0.6 microgram was found to be equal in biological activity to 1 microgram of the 1931 standard carotene. To avoid confusion the biological value of the International Unit of Vitamin A was kept the same, so that it was defined as the biological activity of 0.6 microgram of the 1934 standard carotene. This is now known to be only ninety per cent. pure [311]. The confusion which may arise from defining the unit of vitamin A in terms of carotene is discussed on p. 13.

In America different units for vitamin A have been adopted, based on the Reference Cod Liver Oil of the United States Pharmacopœia; such units are known as U.S.P. units. The original intention was to make one U.S.P. unit equal one International Unit, but it now appears probable that 100 U.S.P. units only equal 87 I.U. [337]. The first Reference Cod Liver Oil in theory contained 8,000 I.U. per gram, but in 1942 a second Reference Cod Liver Oil was prepared which, after assay against the first oil, was given the official value of 1,700 U.S.P. units per gram [338].

Other units of vitamin A have been employed, but their value is slight because they cannot be converted into International Units.

The Sherman, or Sherman-Munsell Unit, was a widely used biological unit based on that amount of vitamin A necessary to maintain a gain of 8 grams a week in a standard test rat over a period of from four to eight weeks [58]. Efforts have been made to convert Sherman units into International Units, but this is impossible, since Sherman units can have no absolute value. However standardized are rats and the conditions under which they live, they will grow with the same supplement of vitamin A at different rates at different times. So a Sherman unit will fluctuate with this fluctuation in the growth response of the animals, which can only be ruled out when a standard vitamin preparation is used for reference at the same time as the estimation is being made (p. 8).

Lovibond blue units, or blue units, or the blue value, are based on comparing the blue colour of the Carr-Price reaction with graded Lovibond glasses. There is no way as yet of converting blue units to International Units, but this is because up till now various factors have altered the values, such as the age of the oil being estimated (p. 6). When these factors have been ruled out, conversion should be possible. At present blue units need to be multiplied by a figure between 20 and 40 to be converted into International Units per gram [59]. One "blue unit Moore" equals about 0.6 I.U. per gram [90]. With [310] has reviewed the various units and their relative values.

PHYSIOLOGY OF CAROTENE OR PROVITAMIN A

Vitamin A is formed in the body from certain of the red or yellow plant pigments known as carotenoids. No animals can apparently make carotenoids for themselves, nor form vitamin A from any other source [76]. Only those carotenoids, however, which contain an unsubstituted beta-ionone ring can be converted into vitamin A (p. 4); of these there are

four which are important in animal nutrition, alpha-, beta- and gamma-carotene and cryptoxanthin. Echinenone, a carotenoid found in sea urchins [60], and myoxanthin and aphanin, found in algæ, can also be converted to vitamin A [76]. All these provitamin A carotenoids are generally collectively referred to as carotene. Morton [871] gives an excellent and comprehensive account of the carotenoids.

Carotene is widely distributed, being found both in bacteria [61] and the higher plants. Its value to plants is obscure, but since it is generally found associated with chlorophyll in green leaves it appears to be important for photosynthesis. In animals its only known value is to act as provitamin A, though its presence in the corpus luteum and suprarenal glands suggests it has some function of its own, and it is said to protect rats against anoxia [180].

As the International Unit of vitamin A is defined as the amount which has the same biological activity as a certain amount of carotene (p. 9), it might appear that vitamin A and carotene are synonymous from the point of view of biological activity. This, however, is not so except when the diet contains very little of both carotene and vitamin A, since in diets which are not frankly deficient carotene is neither absorbed so readily nor utilized so well as vitamin A: the body tends to be wasteful of carotene.

Absorption of Carotene. In man reports differ as to the amount of carotene absorbed from various foods; Wilson [77] found that eighty to ninety per cent. of the carotene in spinach was absorbed when added to a normal diet, but Booher [78] observed that the carotene of spinach was a poor source of vitamin A, being only about half as effective as that of green peas. She also found that carotene dissolved in cotton-seed oil was only half as effective unit for unit as vitamin A in cod-liver oil [79], which is confirmed by sixty per cent. of such carotene being excreted in the fæces [315]. Kreula [80] reported that only twenty per cent. of the carotene of raw carrots was absorbed and five per cent. if the carrots were cooked; these amounts were not increased for one vegetarian who might have been expected to have developed a greater economy in his absorption. Other workers [333] report one per cent. of raw carrot is absorbed, which increases to nineteen per cent. after cooking. After carotene is eaten its absorption from the intestine continues for two or three days [120, 200], and its excretion in the fæces for a week [315]. Studies of carotene absorption based on the amount excreted will tend to give too high values for absorption, large amounts being destroyed in the gut [369]. (See also p. 45).

The placental barrier is not easily passed by carotene. In newborn infants the level of carotene in the blood is only about one-fifth [422] or one-tenth [424] of that in the maternal blood, though there appears to be some definite relationship between the two [424]. Neuweiler [414] has given figures for the amounts of carotene in the placenta and blood of newborn infants. In rats a diet rich in carotene during pregnancy does not increase the foetal hepatic stores of vitamin A, and it appears probable that calves also obtain very little carotene before birth; in the latter the colostrum compensates for the impermeability of the placenta to carotene [99]. In considering foetal nutrition it is essential to remember that it is

profoundly affected by the different types of placenta found in different species, and that the secretion of colostrum is not the beginning of lactation but the end of foetal nutrition; Needham's extremely important book should be consulted for a discussion on this subject [426]. (See also pp. 15, 57.)

Fat is necessary for the efficient absorption of carotene. Wilson [77] reports that in men on a fat free diet the absorption of carotene is nearly halved, and Heyman [66] attributed the poor absorption of carotene during toxic fevers to the poor absorption of fat, which is further born out by his finding no absorption in a child with coeliac disease. Clausen [200] also reports that fever and diarrhoea decrease carotene absorption. Increasing the fat in the diet above thirty per cent. does not, however, increase the absorption of carotene [78]. Ahmad [62] found that in animals only ten to twenty per cent. of carotene was absorbed when it was dissolved in ethyl laurate, and added to a fat-free diet, this amount rising to eighty or ninety per cent. when ten per cent. of fat was also taken. Majumdar [65], however, reports that colloidal carotene in a glucose solution is well absorbed from fat-free diets. Probably the importance of fat is due to its dissolving the carotene and so presenting it for absorption in a fine emulsion.

Bile is necessary for the absorption of carotene, since in animals after ligaturing the common bile duct, or short circuiting it into the colon, carotene is not absorbed by mouth unless it is given with bile salts [67], while Irvin and others [279] have shown by using isolated intestinal loops that lipase is as important as bile.

Liquid paraffin seriously interferes with the absorption of carotene by dissolving it from the food in the intestine, with the result that it is excreted with the paraffin in the faeces. Curtis [85] and his collaborators have shown by careful work on man that on a high carotene diet the carotene level in the blood only rises to half the normal value when 20 c.c. of paraffin are taken thrice daily after meals; taking it twice daily has nearly as bad an effect, and even taking it once at night has some effect. Emulsions of paraffin and agar-agar acted in the same way as paraffin. Similar results have been obtained in work on animals [86, 279], so that when giving solutions of carotene the solvent must not be paraffin but some saponifiable oil, either coconut oil [51] or better still an oil like soya bean oil, which contains vitamin E as an anti-oxidant (p. 717).

Carotene is not excreted in the urine, but when present in abnormal quantities in the blood (p. 78) is excreted by the skin [386, 388].

The transfer of carotene across the wall of the gut probably depends, according to Drummond and MacWalter [87], on the formation with bile acids of a water soluble diffusible complex. Having passed into the lacteals from the intestine the carotene is transported to the liver in the form of a colloidal suspension [88]. It has been suggested the carotene in the blood has to form a complex with protein to remain in suspension, but this does not appear to be necessary since aqueous colloidal suspensions behave in the same way as a carotene protein complex [87]. Carotene undergoes no change during intestinal absorption since it is active when given by

inunction through the skin [128] or injected into the tissues, though much is destroyed at the site of the injection [315]. After injecting carotene into the circulation it can be seen stored in the Kupffer cells [89], from which it gradually disappears.

Conversion of Carotene to Vitamin A. The efficiency of the conversion of carotene to vitamin A within the body is surprisingly poor. From the structure of the β -carotene molecule (p. 8) one would expect it to be split symmetrically into two vitamin A molecules, but since β -carotene is only half as biologically active weight for weight as vitamin A, fission in the body must be asymmetrical, giving only one molecule of vitamin A and decomposition products [68, 76]. The other provitamin carotenoids, since even in theory they can only form one molecule of vitamin A (p. 4), are even less biologically active than β -carotene [76, 311]. The inefficient conversion of carotenoids, however, may be exaggerated owing to their poor absorption from the gut [315].

The conversion of carotene to vitamin A occurs in the liver, by the action of an enzyme called carotinase. Moore's [21] classical paper in 1929 first suggested this, since on feeding vitamin A depleted rats with carotene vitamin A reappeared in the liver. Incubation of carotene with fresh liver tissue or aqueous extracts changes the carotene to vitamin A; this conversion is stopped by heat, which suggests the action of an enzyme [33]. This has been confirmed by removing the liver of oxen, plunging portions into paraffin wax, and allowing aseptic autolysis to proceed, when the carotene of the liver decreases and the vitamin A increases. This conversion again appears to be dependent on enzymic activity, since the liver cells themselves must have died in the anaerobic autolysis. Neither the spleen nor kidney during similar autolysis convert carotene to vitamin A [32]. The contradictory results of Ahmad, who perfused cats' livers with carotene for nearly eighteen hours but found no conversion, are explained by the cat never being able to utilize carotene even when it is added to a vitamin A deficient diet [62].

Whether it is the hepatic cells or the Kupffer cells of the reticulo-endothelial system which convert carotene to vitamin A is uncertain.

Popper [290], using fluorescence microscopy, states that the Kupffer cells and the endothelial cells of the lung and kidney convert carotene, but Wilson [32] could show no conversion by either the kidney or the spleen. Popper explains the poor conversion which occurs in phosphorus poisoning [92], diabetes (p. 51), myxœdema (p. 39), general toxæmias such as uræmia (p. 88) and hepatic diseases (p. 54), as being due to the toxic hepatic cells being unable to take the vitamin A from the Kupffer cells. This leaves the latter so congested with vitamin A that they cannot convert any more carotene.

Utilization of Carotene. Moore [82] found that in animals small amounts of carotene added to diets deficient in vitamin A were utilized as well as vitamin A, but that increasing the carotene decreased the percentage stored, till at very high intakes only about one to two per cent. was converted into vitamin A; five to ten per cent. was lost in the faeces, the remainder apparently being destroyed in the body [88]. He also states

that it is impossible to cause the symptoms of over-dosage with vitamin A by giving carotene. Gray [84] reported that in rats at levels of intake above the minimum to prevent symptoms of a deficiency, vitamin A was six to ten times better utilized than carotene, while Guilbert and others [212], and English workers [832], have also emphasized that at optimum levels of intake 1 I.U. of vitamin A is equivalent to about 8 I.U. of carotene. Children utilize carotene very badly [309]. It has already been mentioned above that cats cannot use carotene as a source of vitamin A. From all this it appears to be certain that carotene is a far less satisfactory source of vitamin A than is vitamin A itself (see also p. 50).

PHYSIOLOGY OF VITAMIN A

Sources. Herbivorous animals depend entirely on carotene for their vitamin A. Omnivorous animals like man obtain vitamin A partly from carotene, partly from animal foods in which the vitamin itself is present. Some purely carnivorous animals like cats are unable under normal conditions to convert carotene to vitamin A [62]. How fish acquire such large stores of vitamin A in their livers, or of what use it is when stored, is obscure, though in some fish it may possibly be necessary for the transference of fat across the gut wall [271], though this does not appear to be so in animals [843, 898]. The seaweeds and plankton, which form the basis of marine food, contain carotene, but the small fish and crustacea which act, as it were, as intermediaries between the plankton and the larger fish do not contain vitamin A or carotene, though they do contain carotenoid pigments. The fish may use these as precursors of vitamin A, carefully storing it up, so that it increases in amount in the liver with increasing age [63], or they may themselves apparently synthesize the vitamin [64].

Absorption. The transfer of vitamin A across the gut wall is in the form of the alcohol, the naturally occurring vitamin A esters being first hydrolyzed, like the other esters of the fatty acids, by the enzymes of the gut [96]. Before passing into the lacteals the alcohol is again combined with fatty acids, since in the chyle from a patient with a chylous fistula Drummond and his collaborators [88] found the vitamin in the form of an ester. As all the vitamin A esters found in the rat's liver appear to be very similar as regards their fatty acids it is probable that there is a selective utilization of certain fatty acids for esterifying the vitamin [84]. Vitamin A undergoes no change during intestinal absorption, since it is active when given by inunction through the skin [128] and is as effective when injected as when given orally, though for injection the solvent is most important; propylene glycol, but not cod liver oil, being satisfactory [860]. Peck [481], however, states that massive doses of vitamin A when injected lower the blood level and Steigmann and Popper [448] found little or no effect. Since vitamin A given by injection has been frequently used with success in conditions such as xerophthalmia and in research work, it would seem that it is the excessive quantity and not the way it is given which accounts for the findings mentioned above.

Absorption from the gut is rapid and complete [815], the maximum rise in the level of vitamin A in the blood occurring within three to five hours of its being taken by mouth [119, 120, 215]. The level of vitamin A in the blood is discussed on p. 56.

The placental transfer of vitamin A to the foetus appears to be controlled by various factors which have not been fully investigated. Byrn and Eastman [422] found the average level of vitamin A in the blood of fifty newborn infants was 91.8 I.U. and that of the mothers 106.8 I.U., but there was no constant relationship and wide differences occurred; this has been confirmed in 143 mothers and children by Lund and Kimble [424] who also noted that the foetal blood vitamin A might be higher than the mother's when this was low, and further that the levels in a pair of identical twins were 30 and 71 I.U. per 100 c.c. Human foetuses and infants tend to have low stores of vitamin A (p. 17). In rats, but not in cows [441], foetal stores cannot be markedly increased by giving very large amounts of the vitamin in the maternal diet; the addition of fat slightly increases storage [99]. (See also pp. 12, 57.)

Factors Influencing Absorption. *Bile.* In animals the presence of bile is unnecessary for the absorption of vitamin A, since Greaves [67] found that ligaturing the common bile duct or anastomosing it with the colon did not interfere with absorption; but Breese and McCoord [98] and others [418] state that children with catarrhal jaundice have a poor absorption of vitamin A which is assisted by bile salts given by mouth. Signs of a vitamin A deficiency have been noted post mortem in children dying from congenital atresia of the bile ducts [94], which was probably not due to the jaundice, since jaundiced animals utilize vitamin A [67], while clinical work also suggests the importance of bile (p. 55).

Fat. Until recently all vitamin A preparations have contained oils or fats, so that there is no experimental work on how vitamin A is absorbed when given quite apart from fat, but the higher biological value of vitamin A as an ester than an alcohol (p. 9), if confirmed, suggests the fatty acids aid absorption. Rough clinical proof that fat, in normal amounts at least, aids absorption is given by the large number of investigations which show that in all diseases where the absorption of fat is impaired the absorption of vitamin A is also impaired (p. 52), though decreased intestinal motility in these diseases may also play a part, since cascara and prostigmin increases absorption of the vitamin in fibrocystic disease of the pancreas [862], while in normal subjects atropine decreases it [870]. The importance, however, of fat for the absorption of vitamin A is academic as regards foods, since vitamin A is always associated with fat, but the small amounts of fat in highly concentrated medical preparations of vitamin A may decrease their value. Impure lecithin, containing also cephalin and inositol, when given in 10 gm. doses with vitamin A, roughly doubles the subsequent rise in serum vitamin A compared to that from vitamin A without lecithin [861].

Liquid Paraffin. In animals liquid paraffin does not interfere with the absorption of small amounts of vitamin A [86], but in man Anderson [118] found that when liquid paraffin was given with large doses of

vitamin A about a quarter was excreted with the faeces, being dissolved

in the paraffin; one patient, for instance, when given 20,000 I.U. by mouth normally excreted 800 I.U. in the faeces, but this amount rose to 5,000 I.U. when liquid paraffin was taken, so it should not be used as an aperient [373].

Digestive Diseases.

Diseases of the digestive tract do not appear greatly to affect absorption, since Moore's [90] figures for vitamin A reserves in the livers of adults dying with gastric ulcers are moderately high, and with enteritis and colitis not very low compared to deaths from some other diseases; in China diarrhoea and dysentery are reported to cause lower reserves [95]. Lerner and Rapaport [121] only found symptoms of vitamin A deficiency, as shown by poor dark adaption, in forty-one per cent. of thirty cases of chronic ulcerative colitis, as against twenty per cent. in other patients in the hospital. In this condition Page and Bercovitz [363] report that absorption is inversely proportional to the number of stools and that the level of the serum of vitamin A is generally normal. The effect of diseases in which fat absorption is impaired

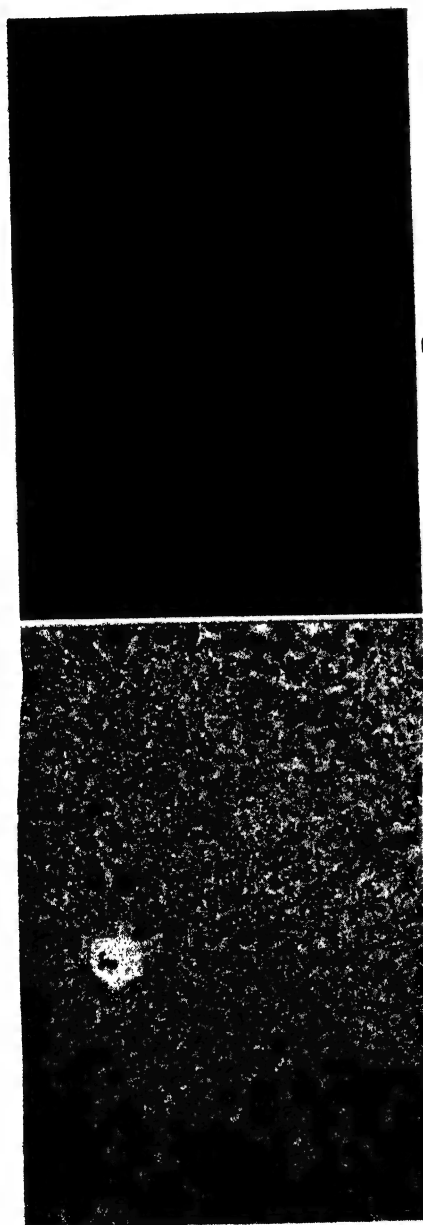


FIG. 2. Photomicrographs of a biopsy specimen from the liver of a patient with peptic ulcer without damage to the liver. A, routine histologic examination: normal structure of the hepatic parenchyma. B, examination with fluorescence microscope: vitamin A fluorescence imparted by fine lipid droplets at the edge of the liver cells and in the Kupffer cells. (See also Figs. 11 and 12.)

is discussed on p. 52, and the effect of hepatic disease, of fever and of intestinal parasites, on pp. 52, 54, 55.

Storage. Storage of vitamin A is confined to the liver for all practical purposes (Fig. 2), Moore [97] finding in his early work that the liver of rats on a high carotene diet contained 100,000 times the amount of vitamin A present in the storage fat of the body, while no other organs contained any. Later work with Davies [88], however, showed that with moderate stores in the liver small amounts of vitamin A were always present in the kidneys and sometimes in the lungs. When the diet was very rich in vitamin A, so that the liver stores were five per cent. of its dry weight and could in theory have lasted the rat for 200 years, the lungs stored more than the kidneys, the amount being larger in both organs than those found in normal livers. The suprarenal glands also, but inconstantly, stored vitamin A in large amounts, while all the other tissues of the body contained traces. In man the adrenals, testes, and ovaries—from infancy to the climacteric [364]—and the lactating breast are all said to store vitamin A, but the kidney does not unless nephritis is present [290]. Moore and others [298], however, have shown that it is the healthy human kidney which stores vitamin A: in nephritis and respiratory diseases the kidney generally contains none. Tumours only store vitamin A if it is present in the parent cells and the retina if it is light adapted [290]. The level of vitamin A in the blood is discussed on p. 56.

The normal storage in human adults from the ages of fifteen to fifty-nine is given by Moore [90] as being 220 I.U. per gram of wet liver; other workers report higher values [313, 412]. For twelve healthy children between the ages of four months and four years the average was 180 I.U. [90]. In infants who died from various causes the reserves in International Units were found to be from birth to one month 17, one to three months 14, four to eight months 100, nine to eighteen months 73, nineteen to thirty-six months 99 [98]. These figures are very low for the younger infants, even allowing for the fact that the conditions from which they died may have caused considerable depletion. Popper [290] also found very low stores in young infants, but Lewis and others [423] report that the average in twenty-four infants who died at birth was 134 U.S.P. units per gram. In the five months' embryo the stores of vitamin A are reported to be high [290]. In the young of animals the reserves of the suckling vary with the maternal diet [99], and since pregnant women are often deficient in vitamin A (p. 63) the low reserves of infants are probably in part explained by their mother's diets. Braun and Carle [441] have reviewed the literature on infants and animals.

Both the hepatic cells and Kupffer cells store vitamin A [290, 312], so that in phosphorus poisoning of the hepatic cells good stores remain [89, 90] while "blocking" the Kupffer cells reduces storage [91], presumably by both decreasing storage space and carotene conversion. The Kupffer cells are the last cells to give up their stores of vitamin A, and the first to replenish them [290]: this is shown in Figs. 11 and 12.

Factors, apart from Intake, affecting Storage. Disease. Moore [90] gives the following figures, in International Units of vitamin A per gram of wet liver, for adults dying of various diseases: thyrotoxicosis 310, diabetes 300, poisoning 170, hypertension 120, conditions of the gall

bladder 110, gastric ulcer 110, coronary thrombosis 110, tuberculosis 96, syphilis 95, endocarditis 90, bronchiectasis and bronchitis 80, subacute nephritis 75, peritonitis 75, enteritis and colitis 74, meningitis 78, pneumonia 68, empyema 60, valvular disease of the heart 60, septic diseases 51, prostate 40, chronic nephritis 25, kidney and bladder infections 19. Moore also quotes Wolff who found very low reserves in syphilitic cirrhosis of the liver which has been confirmed in other forms of cirrhosis [412] and hepatic disease [290]. (See Figs. 11 and 12.)

In children under the age of fifteen [98] the values were: tuberculosis 140, measles 110, pneumonia 78, meningitis 68, septic diseases 47, heart disease 15. Some of the children who died from measles had been given large amounts of vitamin A, but by comparison with other children not given vitamin A it appears that they only had stored about seven per cent., a figure comparable to the storage of only ten per cent. reported by Woo and Chu [95].

A disease may affect storage by decreasing absorption, increasing utilization, hindering the conversion of carotene to vitamin A, impairing the storage capacity of the liver or increasing excretion by the kidney. All or any of these factors may have been the cause of the figures given above, but it may be tentatively suggested: (1) that all the diseases where low storage occurs would tend to decrease absorption by a poor appetite, fever (p. 52), and an unhealthy gut; (2) that the low figures found in heart disease are partly due to anoxæmia interfering with conversion, and partly to the engorgement of the liver with blood which decreases the proportion of vitamin A to liver weight; (3) that the yellow liver found in patients dying from the uræmia of chronic nephritis is due to the presence of carotene, which presumably could not be converted to vitamin A because of the toxic condition of the body; (4) that though many of the pulmonary infections with a low storage are similar to those from which vitamin A deficient animals die, yet the low storage is not the cause of the infections but the result of poor absorption or poor conversion, and increased renal secretion, all of which might then tend to set up a vicious circle by decreasing the power of resistance of the lungs (p. 28).

Vitamin E. The sparing effect which vitamin E has on the metabolism of vitamin A may have more than academic importance *since when human diets contain insufficient vitamin A or carotene an increase in the consumption of vitamin E would decrease this insufficiency* [369].

Further, when vitamin A or carotene are being assayed biologically, equal amounts of vitamin E must be included in the diets of the experimental and control animals, as the response to vitamin A will be affected by the amount of vitamin E in the diets [369].

Davies and Moore [345] first suggested that the reason why vitamin E spares hepatic stores of vitamin A [814], is that it protects them against oxidation within the liver. This idea has been amplified by Hickman and his collaborators [341, 369], who believe that vitamin A in vitamin E deficient animals is drained away from the liver to replenish the blood vitamin A which is lost through oxidation within the blood vessels of the gut. This oxidation is due to oxidants from within the lumen of the gut

diffusing into the blood vessels; normally such oxidants are destroyed within the gut by the vitamin E of the food, which thus also preserves vitamin A and carotene from oxidation before absorption. (Compare the destruction of vitamin E by rancid fat, p. 729.)

The original work which drew attention to the relationship between vitamins A and E was done by Moore [100, 348] and by Bacharach [101]. The former found that the storage of vitamin A—given weekly as halibut liver oil—in vitamin E deficient rats, was increased from two to ten times when the deficiency of vitamin E was removed by giving large amounts of wheat germ oil daily or synthetic vitamin E alcohol weekly. Vitamin A storage from carotene was not so greatly affected. Bacharach [101], keeping rats for shorter periods than Moore on a vitamin E deficient diet, found storage could be increased from twenty-five to thirty-three per cent. by giving large amounts of synthetic vitamin E ester daily; physiologically adequate amounts of the ester had no effect.

In both the above experiments the intake of vitamin A was high; Hickman and his collaborators [341, 369] investigated the subject further by feeding vitamins A and E in small and varying amounts to rats. Their extensive work should be read in full, their chief conclusions being: vitamin E increases the growth-promoting power of vitamin A and carotene and the survival and depletion times of vitamin A deficient rats; there is an optimum ratio between vitamins A and E in the diet, which was also noticed by Moore [100]; vitamin E is most effective when fed together with vitamin A; injections of vitamin E are ineffective; the effect of injected vitamin A is enhanced by oral vitamin E; a mixture of the three naturally-occurring tocopherols is slightly more effective than α -tocopherol alone and much more effective than tocopherol esters; the action of vitamin E is enhanced by reducing agents such as vitamin C. In man vitamin E does not affect the vitamin A tolerance curve [448].

The Essential Unsaturated Fatty Acids or Vitamin F. Quackenbush and his collaborators [339] report that carotene dissolved in ethyl linoleate is unstable *in vitro* and is ineffective in curing vitamin A deficient rats unless protected against oxidation by vitamin E or hydroquinone. This is contradicted by Sherman [342], who states that carotene is stable in ethyl linoleate even when both are incubated with the contents of the rat's stomach, and ethyl linoleate does not decrease the amount of carotene in the faeces. Sherman [297] therefore believes that the inhibitory action which the esters of linoleic and linolenic acids have on the metabolism of carotene occurs after absorption even though he reports that this inhibitory action does not occur if the esters are fed six hours after the carotene [342] or vitamin E is given [297]. On the balance of evidence it would appear that the unsaturated fatty acids only disturb the metabolism of carotene by oxidising it before it can be absorbed: a subject which has been discussed in the preceding paragraph on vitamin E.

Choline. Popper [290] reports that in rats on a diet deficient in choline with ample carotene no vitamin A is stored in the hepatic or Kupffer cells, though it is present in abnormally large amounts in the kidney. When vitamin A itself is given in the diet the Kupffer cells but not the hepatic

cells contain vitamin A. Other workers [442] have thought that a deficiency of choline may hasten the depletion of hepatic stores of vitamin A. The relationship of vitamin A to the other vitamins is described on p. 42.

Other Dietetic Factors. Dann and Moore [102] observed that in rats extreme wasting from lack of vitamin B₁ had no effect on the storage of vitamin A, and beriberi in man does not cause night blindness [307]. Moore [90] found that in man there was no relationship between the stores of vitamin A and the general nutrition. Storage in animals is increased by a high fat diet [103], and not affected by a low protein diet [100]. In rats [442] and chickens [104] a deficiency of vitamin K has no effect on storage. Carcinogens may or may not cause depletion of vitamin A in the liver [308], and depletion is not hastened by 4-hydroxycoumarin, carvone, dimethylaminoozobenzene or flushing out hepatic fat [442]. The factors influencing absorption, and so storage, have been discussed on p. 15.

Mobilization. The mobilization of vitamin A from the stores in the liver and its liberation into the blood is not yet clearly understood. The liver under normal conditions apparently maintains the level in the blood until its own stores are exhausted [393] and can be stimulated to liberate vitamin A by alcohol (p. 52), adrenaline and sympathetic stimulation (p. 68). Other drugs which have so far been tested (p. 68) have no effect. During the first few days of life (p. 57) and during fever (p. 52) and in hepatic disease (p. 54), mobilization is poor or virtually does not occur. The mechanism by which a balance is maintained between the liver, the blood and the urine is altered in favour of the urine and sometimes the blood in renal disease (p. 39) and in conditions where the level of lipoids in the blood is high (p. 41).

Excretion and Destruction. Lawrie, Moore, and Rajagopal [298] in 1941 reviewed the work on excretion of vitamin A by the kidney and also gave the results of their own research. The following is chiefly based on their paper. Vitamin A is never excreted by man during good health, except possibly as a breakdown product [103], but it may appear in the urine during illness, being found most frequently and in the highest concentrations in pneumonia. A daily output of 3,200 I.U. has been recorded, which is said to cease abruptly with the crisis. In chronic nephritis vitamin A is common in the urine though in smaller amounts than in pneumonia. Still smaller amounts have occasionally been reported in chronic infections, rheumatic fever, skin diseases, diabetes, pernicious anæmia, asthma, cancer and normal pregnancy.

How vitamin A is dissolved in human urine is obscure. It is always associated with protein, but not all urines containing protein contain the vitamin, even though they are capable, unlike normal urines, of taking it up from halibut liver oil. Apparently the excretion of vitamin A in the urine is dependent on a functional abnormality of the liver causing a diminished capacity for absorbing or retaining the vitamin. This alters the equilibrium between the liver and the blood, vitamin A passing to the latter. But the blood and kidneys will only yield up the vitamin to the urine if their capacity to retain vitamin A is impaired. In pneumonia this is frequently so to a marked degree. In nephritis the damage to the

kidney probably leads to an accumulation in the blood of substances which increase the solubility of vitamin A and so partially hold it back from excretion.

The healthy dog constantly excretes vitamin A. It is interesting to note that the level of vitamin A in the dog's blood may be extremely low without causing any signs of a deficiency [299] and that its metabolism appears to differ from that in other animals (p. 42). Rats never excrete vitamin A, even when taken in toxic amounts [83, 298] or when their kidneys are damaged by lack of vitamin E [100], and rabbits only when diseased [298]. The presence of vitamin A in the faeces is presumably due to incomplete intestinal absorption [118, 315].

The large stores which accumulate during a diet rich in vitamin A are depleted with great rapidity if the diet becomes deficient in the vitamin [105, 108], or in vitamin E [314], the depletion being greater than can be accounted for by the needs of the body. Moore [105] suggests that there are two ways in which vitamin A is destroyed, one depending on the normal and economical physiological use of vitamin A in the processes of the body, and the other depending on some mechanism for destroying vitamin A when it is stored in excessive amounts.

Factors Influencing Requirements of Vitamin A. *Age, Weight, Metabolism.* It often appears to be accepted as axiomatic that children and young animals require more vitamins than adults, but the reverse of this is true as regards vitamin A, since the requirements, being proportional to the weight, are higher in adults. This is not accepted by all authorities; Brown [106] in 1940, and Bigwood [107], writing for the Technical Commission on Nutrition for the League of Nations in 1939, both state, without giving their reasons, that children need more vitamin A than adults. Harris [108] thinks it is probable because children store less than adults, and more often suffer from poor dark adaptation which is an early sign of vitamin A deficiency (p. 58). But the relatively low stores of vitamin A in children might be taken to mean that the child, like the young fish [63], stores less than the adult because this is physiologically normal, and not because the higher requirements of the body leave little to be stored. Comparisons of the needs of children and adults based on the commonness of poor dark adaptation at different ages is open to the serious criticism that poor dark adaptation may develop in children but not in adults when both are on a slightly deficient diet; in fact children and adults may show a mild deficiency of vitamin A in different ways. This appears probable since Frazier and Hu [110] and Sweet and K'Ang [111] found that while xerophthalmia was an early sign of deficiency in children, it was a late one in adults, often appearing months after other signs (p. 65), while Goodwin [127] stresses the fact that even in children changes in the skin may sometimes precede any eye symptoms and possibly even poor dark adaptation.

From all the foregoing it appears that the proof that children need more vitamin A than adults is slight and indirect, while the converse is supported by a large body of work. Irving and Richards [112] noted that old rats need more vitamin A than young rats both to prevent deficiency

lesions only demonstrable at autopsy, and to keep the development of the continuously growing incisor teeth normal. Guilbert and his co-workers found that in cattle, sheep and swine [118] and also in rats [114] the carotene and vitamin A requirements were dependent on the weight alone, being 25 to 30 micrograms of carotene, or 6 to 8 micrograms of vitamin A per kilogram of body weight, and they suggest this figure holds true—apart from carnivores—for all animals, including man [212] for whom it is in agreement with requirements based on clinical work (p. 49). They also state that the requirements are not affected by the amount of energy expended by the animals, their work being confirmed both as regards the effect of weight and increased metabolism by Guerrant and others [115], who found that when rats on a deficient diet were made to exercise they gained less weight but developed less severe deficiency symptoms than control animals. These results confirm Wolbach's [116] suggestion that vitamin A is necessary not for the metabolic activities of the cells, but for the maintenance of the structure of the cells, so that the requirements of the body would depend on its weight, that is its number of cells, and not on its activity. The changes in metabolism resulting from a low protein diet [100] and a high fat diet [129] do not influence, respectively, storage and consumption while thyrotoxicosis in man (p. 39) appears to increase storage.

All these investigations which run counter to the belief that the young need more vitamin A than the old have admittedly been done on animals, but it is generally believed that the effect of vitamin A is broadly the same in all species [117], so it seems most probable that increasing age and so increasing weight raises the requirements of vitamin A, but that youth, exercise and a raised metabolism have no effect.

Sex. Sex does not greatly influence the requirements of vitamin A, since in countries where xerophthalmia is common both sexes are equally affected [111], and in England Harris [109] found poor dark adaptation equally common in boys and girls between the age of eleven and twelve, though between the age of twelve and thirteen girls were slightly less affected than boys. Kodicek and Yudkin (p. 56) also report that in girls between the ages of eight and eleven the conjunctival epithelium is less frequently abnormal than in boys, though it is emphasized that this abnormality may not be related to a deficiency of vitamin A. Coward [365] after many years work on rats has shown that the male requires more vitamin A than the female, and she suggests this may explain the higher mortality among human male than female infants. The less uniform requirements of the female rat [52] may possibly be related to the fluctuating vitamin A metabolism which occurs in the ovaries, at least of women [364]. Depletion of hepatic stores of vitamin A occurs more rapidly in the male rat than the female [366].

Illness. If the list of the amounts of vitamin A stored in the livers of patients dying from various diseases is referred to on p. 17, it will be seen that in some diseases—notably in chronic genito-urinary conditions, chronic sepsis, infections of the lungs and alimentary tract and hepatitis—there are low reserves of vitamin A which suggests that, whatever is the

cause (p. 18), the requirements for vitamin A in the diet are increased. Some diseases (p. 52) prevent the distribution of vitamin A from the liver to the blood, and so in these large amounts of the vitamin are required if its normal level in the blood is to be maintained.

Poor Utilization of Carotene. When discussing the absorption and conversion of carotene to vitamin A (p. 11), it was seen that diseases which decrease the secretion of bile, hinder the absorption of fat and food or affect the liver and its metabolism lead to poor absorption and conversion of carotene, so that the requirements of vitamin A itself are increased to compensate for the deficient utilization of carotene.

Action of Vitamin A in the Body. Nothing definite is known about what is the fundamental part played by vitamin A in the metabolic processes of the body: all that can be said is that a consideration of its chemical structure with five unsaturated bonds (p. 3) suggests its probable function as an oxidation-reduction catalyst; while the needs of the body depending on weight, and not on metabolic activity, seem either to imply that it is chiefly necessary for cellular structure rather than function (p. 21), or, as Mason [180] has suggested, it is necessary for some particular metabolic process peculiar to all epithelial cells in varying degrees, but not to other tissues. There is also some evidence that vitamin A has a specific effect on the growth of animals [353] and quickens growth and prolongs the life of tissue cultures [181, 182], which is reminiscent of Batchelder's work [183] where she found that a very plentiful supply of vitamin A throughout the life of the rat increased longevity and delayed the onset of senility.

The Effects of Lack of Vitamin A on the Epithelial Surfaces of the Body.

"The specific tissue change due to deprivation of fat soluble vitamin A is replacement of various epithelia by stratified squamous keratinizing epithelium" (Wolbach and Howe [184]). If this statement is broadened to include all tissues of epithelial origin be they from endoderm, or ectoderm like the skin, nervous system and retina, a valuable generalization is made which harmonizes all the more important functions of vitamin A, always remembering that some tissues are damaged earlier than others by a deficiency. The epithelial changes are the direct effect of lack of vitamin A and are not the secondary result, as was suggested by Mellanby, of the degeneration of the nervous system which also occurs; the subject is discussed fully on p. 34.

The change in the epithelium is the same in all areas where it occurs, the normal epithelium becoming undermined by stratified epithelium, which starts to be formed by the basal layer of cells in many places at the same time. The basal cells themselves, however, are not changed, maintaining their individual properties, so that when vitamin A is given to a deficient animal the basal cells promptly start to replace the stratified epithelium with epithelium of the correct type.

In considering in detail the changes induced in the various surfaces of the body Wolbach and Howe's [184] description of the rat will be followed, references being given where later work has amplified their observations,

or extended it to other species, while post mortem observations in man are described on p. 77.

Young animals develop symptoms first, due probably to their naturally low stores of vitamin A (p. 17), the most striking features at the time of death being a humped posture, rough fur, emaciation, and encrusted eyelids.

Glands. The epithelium lining the ducts of the glands is involved before that lining the acini, and in general the changes are not very pronounced. The shedding, however, of desquamated cells by the new keratinizing epithelium into the ducts blocks these with the result that retention cysts occur in the glands, and then secretion is impaired even when the secreting cells are not yet affected. This accounts for the early observations on the common occurrence of abscesses or cysts at the base

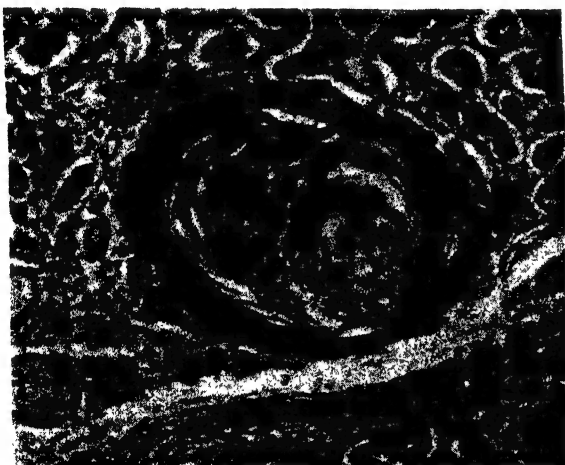


FIG. 3. Pancreas of an American infant showing duct occluded by keratinizing epithelium.

of the tongue, which are really salivary retention cysts that may become infected. As well as all the salivary glands the conjunctival and lachrymal glands are involved, soon and constantly, while the glands of the duodenum are only slightly affected and the pancreas escapes apart from some late changes in the duct. In Wilson and Du Bois' [185] child, however, the pancreas was more affected, the ducts being blocked and the

acini cystic, though the islets of Langerhans appeared to be normal (pp. 28, 77). There is late atrophy of the sebaceous glands in the rat [846], but in man they and the sweat glands may become blocked early in the deficiency (p. 64).

Air Passages. All the respiratory tract, the nasal sinuses, and Eustachian tubes are involved. This is of special importance in the lungs, where the smaller bronchi become plugged with desquamated cells, thus paving the way for bronchiectasis and infection, both in animals and man (pp. 72, 77). The otitis media, however, which is usual in deficient rats does not occur in children [876].

The Eyes. The conjunctiva undergoes constant and early keratinization followed by œdema of the cornea and its invasion by blood vessels from the sclera. Dryness or xerosis of the eye is caused by the drying up of the lachrymal secretion due to the involvement of the lachrymal glands. The latter was held at one time to be the primary cause of the changes in

the conjunctiva and cornea, but Wolbach and Howe showed that xerosis occurred after keratinization, which is supported by clouding of the cornea in the horse [136] and xerosis in man (p. 71) occurring at the same time as an increased secretion of tears. Ultimately the whole cornea softens, and the condition known as keratomalacia occurs which, aided by infection, leads to perforation. The changes observed in man, which are broadly the same, are described on p. 70.

The corneal vascularization which follows the keratinization is, in the rat at least [417], indistinguishable from that which occurs with a deficiency of riboflavin (p. 387). This raises the very interesting question whether the corneal vascularization caused by lack of vitamin A is not really directly due to a local deficiency of riboflavin caused by the absence of ocular secretions, since it appears probable that the cornea is normally supplied with riboflavin not by the limbic blood vessels but by being bathed in the tears and Meibomian secretion, both of these containing large amounts of riboflavin [419]. The rather scant descriptions of the vascularization which occurs in human xerophthalmia appear to have been made when a secondary infection had blurred the picture [418].

Olfactory Epithelium. Loss of smell is a late but constant finding in rats and other animals [376], though the olfactory epithelium appears to be normal [184]. The olfactory nerve endings themselves may be damaged by osseous thickening of the cribriform plate (p. 35). Milas [187] found that the olfactory area in the steer was rich in carotenoids and vitamins A and A₂, which suggests that vitamin A may play a part in smell analogous to that it plays in vision. It would be interesting to know if a deficiency of vitamin A in man causes loss of smell.

Digestive Tract. The mucous membrane of the digestive tract of the rat is not involved, apart from slight changes in the œsophagus, unless the deficiency of vitamin A is very prolonged, when considerable thickening of the mucosa of the forestomach occurs, which does not persist after the deficiency is removed, nor cause the formation of malignant tumours as was once thought [138, 319]. In dogs and rats a deficiency does not alter the secretion of acid in the stomach nor have a definite effect in dogs on the emptying time [189, 321].

In man (pp. 72, 77) severe deficiencies often cause diarrhoea and there is some evidence that vitamin A is important for the function, if not the structure, of the gastric glands and pancreas. Földes and Vajda [280], following up unimpressive German work, report that in twenty cases with deficient or absent hydrochloric acid, twelve improved, both as regards their symptoms and the amount of acid in their test meals, after two or three weeks on 16,000 I.U. of vitamin A thrice daily. The patients who benefited had chronic gastritis, neurasthenia, gastropotosis, diabetes, thyrotoxicosis, or renal lithiasis. The eight patients who did not benefit had gastric carcinoma, pernicious anæmia, or gall stones. One patient in the last group was given bile salts as well as vitamin A, which caused a return of hydrochloric acid to the stomach, even though histamine injections had failed. Herfort [281], however, did not find that vitamin A had any effect in hypochlorhydria, though it did increase the amount of lipase and the

tryptic activity of pancreatic juice not only in normal subjects but also in those with various vague intestinal symptoms; in the latter diarrhoea was decreased and the appetite improved. Preliminary work by Seelig [296] suggested that large amounts of vitamin A rapidly removed both the symptoms and radiological or gastroscopic evidence of gastric ulcers, but work by Douthwaite [368] did not confirm this.

Gums and Teeth. In man lack of vitamin A causes the gums to become hyperplastic and keratinized [140], while the developing teeth both in infants [141] and animals are severely damaged, since the enamel organ



FIG. 4. Incisor tooth of rat on a vitamin A deficient diet for 170 days followed by 14 days with addition of butter fat. Ameloblast inclusions are seen in the dentine. Newly formed dentine has filled spaces between the folds of dentine and has surrounded the inclusions of ameloblasts. The restoration of odontoblasts is shown and heavy calcification of the dentine on the labial side of the tooth, a usual response to restoration of vitamin A to the diet.

being of epithelial origin shrinks, or is replaced by keratinizing epithelium, thus apparently removing from the odontoblasts some controlling influence over their growth. They therefore form poor or deformed dentine, which in prolonged mild deficiencies leads to the formation of odontomas and tooth reduplication in the perpetually growing incisors of rodents [142]: such teeth also lose their colour [345]. Mellanby [142] has shown that in animals lack of vitamin A in the maternal diet can seriously damage the teeth of the young before their birth. It therefore seems probable that vitamin A is the most important vitamin for the structure of the dentine and enamel, in spite of the commonly and quite erroneously held belief that lack of vitamin D is the main cause of dental degeneration (p. 687).

Urinary System. Vitamin A plays an important part in the function of the kidney, which is discussed on p. 87, but lack of vitamin A does not structurally alter the epithelium of the renal tubules, though it causes the usual epithelial changes in the pelvis of the kidney, ureters and bladder in animals; in man changes are seldom so severe (p. 77). There is, however, one important result of the change in the mucosa, since the new epithelium sheds cells into the urinary tract, so providing a nidus round which salts are deposited to form calculi: this occurs frequently in rats and guinea-pigs.

In man, however, it is probable that lack of vitamin A is not a factor in causing renal calculi, though this has frequently been suggested partly from analogy with animals, partly from observations that patients with renal calculi often have symptoms of a deficiency of vitamin A. Thus Long and Pyrah [123] and others [180] have reported that renal calculi are frequently though not always associated with poor dark adaptation, the most chronic cases having the worst adaptation [123]. On the other hand, Jewett and his colleagues [381] compared a group of twenty patients suffering from renal calculi with a group of forty normal people; both groups had equally good dark adaptation and similar levels of blood vitamin A. Post mortems on seventy-eight patients with renal calculi showed no epithelial changes suggestive of a deficiency of vitamin A in either the lungs or urinary tract. It would appear most probable that when urinary calculi and signs of a deficiency of vitamin A occur together it is not the deficiency which has caused the calculi but the calculi which have caused the deficiency, through disturbing the metabolism of the vitamin as a secondary result of the damage they have inflicted on renal function (p. 87).

Genital Ducts and Epithelium. Mason [180] in a very careful study has shown that vitamin A is essential for the germinal epithelium of the testes, its deficiency causing changes unlike those produced either by starvation or deprivation of vitamin E. The earliest changes are sloughing of germinal cells into the lumen of the tubules, with a gradual reduction in the latter's size. As the degeneration becomes more advanced only three or four layers of cells are left lining the tubules, but these still are capable of forming an occasional sperm, and at no time can the testis be so damaged that it cannot return to normal when the vitamin A deficient diet is stopped. These changes are due to a direct effect on the cells themselves and not an indirect one from vitamin A acting on the pituitary, since neither pituitary transplants nor injections of pregnancy urine hastened recovery, and also because the degeneration caused by removal of the pituitary is unlike that caused by a lack of vitamin A. A rather puzzling relationship was also noted between vitamins A and E. When both were deficient the testes sooner showed signs of a vitamin E deficiency than when a deficiency of vitamin E was present alone, though the decrease in the number of cells due to lack of vitamin A might have been expected to decrease the need for vitamin E. It was further found that a vitamin E deficiency when superimposed on an existing vitamin A deficiency did not cause such serious damage as when the vitamin E deficiency occurred

alone. Wolbach and Howe [184] also noted œdema outside the basement membrane of the seminiferous tubules and the usual epithelial changes caused by the lack of vitamin A in the mucosa of the epididymis, prostate, and seminal vesicles and, in the female, in the oviducts, uterus and vagina. The vaginal changes have been used as a guide when doing biological tests for vitamin A, being among the earliest signs of a deficiency (p. 7). The part played by vitamin A in reproduction is discussed on pp. 30 and 41.

Endocrine Glands. The structure, as apart from function (p. 39), of the endocrine glands is said to remain normal; no change beyond decrease in size was noted in the rat's anterior pituitary, thyroid, thymus, parathyroids, suprarenals, islets of Langerhans, ovaries, Graafian follicles, corpora lutea, and interstitial tissue of the testes. In a human infant dying from lack of vitamin A Hassall's corpuscles were found to be enlarged [185].

Skin and Hair. The rough coat of the rat [346] and horse [186] and "toad skin" and absence of sweat in man (p. 63) are due to hyperkeratinization of the epidermis and the atrophy of the hair follicles and the sebaceous and sweat glands, and their blocking by desquamated cells. In man the scalp hair and nails are affected little if at all [110, 111].

Fœtal Epithelia. Maxwell [148] has reported children being born with keratomalacia, and Hale [144] found that farrows born of vitamin A deficient sows were blind, often with no eyeballs, cleft palates, harelips, extra ear-like growths and misplaced kidneys. There seems to be definite evidence that permanent congenital blindness in calves can be caused by lack of vitamin A [145]. Cannon [146] could not cause any congenital abnormalities in rats and Anderson [817] found no changes in the intestinal epithelium, though she reported an increase in congenital diaphragmatic herniæ. Abnormalities in both the soft and osseous tissues have been ascribed to lack of a factor found in liver, rather than to a deficiency of vitamin A [818]. On the whole it seems improbable that human developmental defects are due to lack of vitamin A, especially as in countries like China, where a deficiency is endemic, there are no reports of such defects being unduly common. Straumfjord [241] has suggested that *vernix caseosa* is really due to the fœtal skin being hyperkeratotic as a result of lack of vitamin A in the maternal diet, but this is probably incorrect, since Lund and Kimble [424] found no relationship between the level of vitamin A in the blood of newborn infants and the amount of *vernix caseosa* (see also p. 57).

The Secondary Result of Changes in the Epithelia due to Lack of Vitamin A : Decreased Local Resistance to Infection

Lowered local resistance to infection is the most important result from the changes in the epithelia brought about by lack of vitamin A. The general defence mechanisms of the body, on the other hand, are not impaired so that the name "anti-infective vitamin" is too broad in its implications; vitamin A is only "anti-infective" to the extent that when it is given to man or animals suffering from its deficiency it increases

the power of the epithelial surfaces to resist local infection by bringing them back to their correct and normal condition.

The importance of vitamin A for the local defences of the body has been recognized for many years. Among earlier workers Cramer and Kingsbury [147] in 1924 pointed this out very clearly and emphasized that these local defences were not entirely concerned with bacterial infections, since their animals also were heavily infected with intestinal worms, which is supported by vitamin A deficient rats being more susceptible to infection with trichinosis [200]. Green and Mellanby [10] found that rats on a vitamin A deficient diet all died with mucosal infections, and that the addition of vitamin A, as carotene, to the diet afforded a degree of protection against these infections which was proportional to the amount of carotene added [148]. That the value of vitamin A is purely due to its local effects is shown partly by the infective lesions caused by its deficiency being always epithelial, and partly by observations on the relationship of the humoral defences of the body to vitamin A. Thus Gellhorn and Dunn [149] found that the phagocytic index during the early stages of a deficiency might be increased or decreased, but that after a prolonged deficiency it was always low; they suggest that when the index is raised it is due to the normal reaction to infection, and that when it is low after a prolonged deficiency this is due to exhaustion and is not a direct result of the deprivation of vitamin A, though the index being low sets up a vicious circle which further decreases resistance. Torrance [150] observed that vitamin A deficient animals were no more susceptible to bacterial toxins than were normal animals.

Changes in the blood picture due to deprivation of vitamin A are not so severe as to suggest they would seriously decrease resistance to infection. Wagner [151], studying ten men who took an experimental diet nearly devoid of vitamin A, noted a decrease in the hæmoglobin and erythrocytes, degenerate red cells, a leucopenia with degeneration of the myeloid cells, and a marked fall in the thrombocytes, though none of these changes except the latter were very marked. Abbott and others [152] who diagnosed vitamin A deficiency in 84 children, 45 women, and 28 male students by the condition of their skin and conjunctivæ, and by their diets, found a mild leucopenia with a decrease in polymorphs, a relative increase in large lymphocytes, a decrease in small lymphocytes, with an increase in juvenile and degenerate cells. These findings corresponded to those they had previously obtained in vitamin A deficient rats. That the changes in the blood were due to lack of vitamin A is strongly supported by the blood of all the patients returning to normal after they had taken 51,000 I.U. daily for six weeks. Sweet and K'Ang [111], however, in their very extensive study of vitamin A deficiencies among the Chinese found no alteration in either the red or white cells of the blood, and Hennessey [282] in Uganda reported that in prisoners who were deficient in vitamin A the giving of cod-liver oil did not alter the leucocytic response to injections of a bacterial antigen. The bone marrow of vitamin A deficient rats is normal [170].

Clinical work on the whole bears out that vitamin A is only of value

for increasing resistance to infection when the patients are on a deficient diet and the infection is chiefly concerned with epithelial surfaces ; but observations on man are difficult to interpret since there are few reports which accurately mention whether the patients treated with vitamin A were previously on a good or deficient diet. In the first important clinical trial of vitamin A Green and others [158], having observed that local infections of the uterus and Fallopian tubes developed in vitamin A deficient rats after parturition, gave 275 pregnant women in the last month of pregnancy—when they were presumably deficient in vitamin A (p. 63)—large supplements of vitamin A as “radiostoleum.” Only 1.1 per cent. developed puerperal sepsis, as against 4.7 per cent. of 275 women who had had no extra vitamin A.

Donaldson and Tasker [154] in Johannesburg reduced the mortality

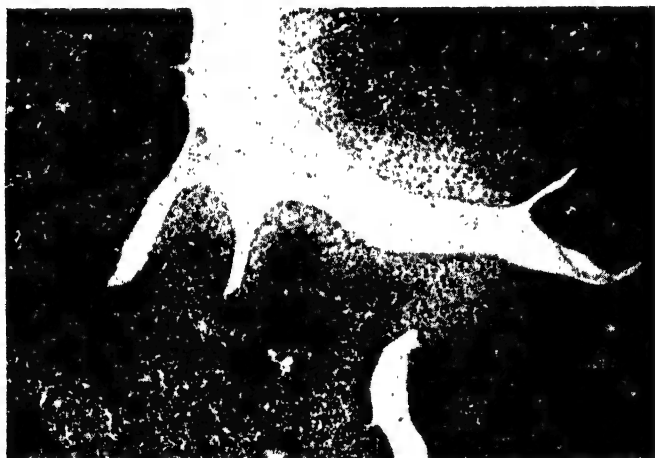


FIG. 5. Uterus of an American infant with epithelium in part replaced by keratinizing epithelium.

from pneumonia among native workers from thirteen per cent. in 100 untreated cases to eight per cent. in 200 cases who were treated with extra vitamin A in the form of “radiostoleum” or liver. Orenstein [155] failed to confirm these results. The effect of vitamin A in whooping cough is uncertain.

Ellison [156] found vitamin A of some slight value in measles, though Mackay and others [157] could not confirm this. Sutliff [159] failed to protect children with scarlet fever from developing otitis media by giving them vitamin A. In typhoid fever in children Giraud and Valette [158] state that vitamin A is of great value for preventing hæmorrhage or perforation of the bowel and skin lesions, but it has no effect on the cardiac and pulmonary complications or the duration of the disease. Other forms of enteritis might be benefited by vitamin A since its deficiency in animals increases not only the number but also the variety of intestinal bacteria [822]. Vitamin A for the treatment of intestinal worms has not been investigated, but should be of value (p. 29).

The "common cold" in its relation to vitamin A has been investigated by many workers. Again the rule appears to hold good that vitamin A is only of value when it corrects its own deficiency. Thus Wright and others [160] in Canada found no effect at all from giving large extra amounts of vitamin A to twenty of sixty infants all of whom already received a desert-spoonful of cod-liver oil daily, and Uddströmer [161] likewise observed no benefit from adding 6,000 I.U. of vitamin A daily to diets which were already excellent. Similar results are reported from America [382], and England [440]. Where the diet is reported to have been reasonably good most observers state that the



FIG. 6. Lung of an American infant with bronchiectatic cavity due to keratinizing metaplasia in bronchi. The cavity is partially lined with keratinizing epithelium.



FIG. 7. High power of Fig. 6.

duration of colds, though not their number, is decreased by additional vitamin A [162] and some workers have reported that in poorly nourished people the number of colds is also less [168]. Vitamins A and D given together are stated to have a greater prophylactic value than either given alone [816].

In infections of the skin vitamin A may be of great value, Ryrie [288] reporting that vitamin A or carotene is almost a specific cure for leprosy ulcers when applied locally, and Banyai [284] obtained good results with cod-liver oil applied to all forms of tuberculous ulceration of the skin, larynx, and pharynx. He also stated that injections of cod-liver oil into tuberculous empyemata, glands, epididymes, and ischiorectal abscesses were of value, but the benefit was probably due to the oil and not its vitamin A (p. 792). Infants fed on roller dried milk supplemented with vitamin A had a decreased susceptibility to minor skin infections in one very thorough investigation of Mackay's, but she found in a second investigation that extra vitamin A had no effect either on the skin, or the general health and immunity to infection, from which she infers that some dried milk may be adequate in vitamin A, but that supplementing it is wise [157]. Thirty cases of senile vaginitis had their symptoms and the changes in the mucosa improved by Simpson and Mason [285] who gave cod-liver oil by mouth. Skin diseases and vitamin A are discussed on p. 68.

Retinal Metabolism and Night Blindness (*Hemeralopia, Nyctalopia or Delayed Dark Adaptation*). Vitamin A is essential for vision in dim light. Even with a mild deficiency the rapidity of dark adaptation in most people and its extent in all people is impaired [875], while a severe deficiency leads to night blindness or hemeralopia. The subject is important partly because night blindness is a grave drawback to countrymen or fishermen working in the dusk or by moonlight and to town dwellers during "black outs," partly because poor dark adaptation is a very early symptom of a deficiency which is widely used in nutritional research on vitamin A, being more easily and rapidly investigated than, for instance, the actual amount of vitamin A in the blood. For a full discussion on the physiology of vision the excellent review by Lythgoe [164] should be consulted; here only enough can be said to explain the commonly accepted rôle of vitamin A in vision. (Clinical applications are discussed on pp. 58 and 63.)

In bright light vision is carried out by the cones of the retina, which probably are not affected by lack of vitamin A, though this is not certain [164, 165]: in dimmer light lack of the vitamin generally decreases the sensitivity of the cones (p. 58). When illumination is suddenly decreased so that there is only about as much light as is given by a three-quarters full moon, vision is impossible for a moment and then "dark adaptation" occurs, the eyes "growing accustomed to the dark." For about the first six minutes of this adaptation the increasing power of vision is due to an increasing sensitivity of the cones to a poor light, but after this further adaptation, which may not be complete for an hour or even much longer and may lead to an increase in sensitivity of 10,000 fold, is due to vision being carried out by the rods of the retina, these becoming sensitive to

illuminations which never can stimulate the cones. In fact very broadly speaking the cones are used in bright light and the rods in poor light.

The rods, however, are not directly stimulated by light, but only indirectly through the chemical changes light causes in visual purple. Visual purple is a complex substance of which vitamin A is a component, and it is always found round or possibly in the walls of the rods. Since it is bleached or destroyed by light the eye contains little after being exposed to bright light, and so has to reform it before it can be utilized by the rods for vision in dim light. On the rapidity with which the visual purple is reformed must depend the rapidity with which dark adaptation, that is, full use of the rods, can occur. Since vitamin A is a necessary part of visual purple any shortage of vitamin A must slow down the formation of visual purple and so slow down dark adaptation. Since even the dim light in which rod vision is used destroys some visual purple, the amount in the retina will depend on the relative rates of destruction and formation. This is the reason for shutting one's eyes for a minute on coming from a brightly lit room to a dark street; by shutting out all light the visual purple can accumulate more rapidly. In the same way badly fed slaves used to see better in the dawn after a night in the dark than at dusk after a day's work in the light. For centuries poorly fed fishermen have known that a day's exposure to glare from the water often causes sudden night blindness—in other words, prolonged bleaching destroys so much of the visual purple that the vitamin A deficient eye cannot reform it in sufficient amounts to give even poor night vision. On the other hand, dark adaptation cannot be improved beyond the normal, however much vitamin A is taken [824]. Vitamin A is present in the light adapted but not the dark adapted retina [290].

Two other factors, besides vitamin A, influence the formation of visual purple. Firstly there must be an adequate supply of oxygen to the retina, and secondly visual purple is regenerated more rapidly—as far as the supply of vitamin A permits—if the retina has been previously exposed for a long period to a bright light. The latter fact may be of importance in clinical work on dark adaptation, though it appears to be generally ignored. Riboflavin may also possibly play a part (p. 62).

There are many clinical reports that delayed dark adaptation or even severe night blindness can be cured in a few hours with vitamin A, while others state it may take weeks or months. It is possible that the latter were also having to cure some further defect from the vitamin A deficiency, such as nervous degeneration in the retina, or in the rods themselves if visual purple is an integral part of their structure and so, by its absence, causes structural damage [874]. There is also every reason to believe that dark adaptation is dependent on fine readjustments in the nervous system of the retina once sufficient visual purple has been formed. Thus while the amount of visual purple during adaptation may be only doubled sensitivity may increase 10,000 fold, which appears to be only explicable if a synaptic rearrangement of the nervous elements of the retina allows each rod and cone to become connected to additional nerve fibres, so allowing for a summation of subliminal stimuli—a theory which is further

supported by the decrease in visual discrimination which occurs during dark adaptation.

Colour Vision. There is some slight evidence that lack of vitamin A impairs recognition of the colour blue [408] and Dunlap and Loken [409] state, in a paper which gives insufficient details of their work, that 25,000 I.U. of vitamin A daily for three to eight weeks usually cure colour blindness. Elder [410], however, after a most careful investigation found no significant improvement in any of forty-one cases. Even if the physiologically surprising fact that vitamin A improves congenital defective colour vision were true, it would be a grave disservice to candidates for the Navy, or other professions in which good colour vision is essential, to mask their disability.

Vitamin A and the Nervous System. The effect of deprivation of vitamin A on the nervous system is still uncertain in animals, while in man all that can be said is that in some nervous diseases, such as lathyrism (p. 78), a deficiency of vitamin A appears to be one of their most important causes. Some workers with animals have found no degeneration of the nervous system when other signs of a vitamin A deficiency were already severe [184, 166]; others working with dogs [171, 291], rabbits [172] and rats [70] have reported a widespread degeneration of the medullary sheaths of the peripheral nerves [167, 170, 172, 173, 174], the optic and auditory nerves [291], the retina [374] and the tracts, chiefly sensory, in the medulla [70] and spinal cord [167, 170, 171]. This degeneration is apparently irreversible [172], though it can be prevented from progressing further with vitamin A [174]. Many observers must have omitted to look for it since it is well advanced before it gives any clinical symptoms [167, 170, 172, 174] and, oddly enough, remains unaltered after the clinical symptoms improve with vitamin A [167]. The explanation of these contradictory results is probably that nervous degeneration only occurs rapidly in young animals (p. 85).

Irving and Richards [70] can produce degeneration of the nervous system with such certainty that, after very careful work, they suggest it should be used as a method of assaying vitamin A, since the difference between the amount of vitamin A which does and does not protect the nervous system is very small. They found degeneration in the medulla was constantly present after seven weeks in young deficient rats, and since, of course, this degeneration must have been present for some time before that and since it occurs in rats that show no other signs of deprivation, it must be among the earliest signs of a deficiency. That inanition alone causes no degeneration has been shown by Aberle [170] and by Wolbach and Bessey [323].

It appears possible that the nervous degeneration is really due to some unidentified factor which is absent in some experimental diets and not others [171], or even more probably, in view of the protective action of nearly pure vitamin A preparations or carotene [169, 172], that the nervous degeneration only occurs when there is a double deficiency of vitamin A and an unknown factor. That the casein, for instance, used in experimental diets contains some factor necessary for growth has been known

for years [52]; while the work of Wintrobe and others [175] on pigs, which in spite of a simplified diet supplemented with nearly every known vitamin still developed a widespread nervous degeneration, further emphasizes how complicated and delicate are the dietetic needs of the nervous system: even copper may be important [327].

Mellanby [291, 376, 377] has reported that in young dogs and other young animals [376] lack of vitamin A causes the compact bone to become cancellous, so that there is overgrowth and an increase in thickness though not in the calcium content. The result is that the brain, and cranial and spinal nerves are compressed; the hind brain, the optic, auditory and olfactory nerves, and the spinal nerves in the cervical region being most affected. In adult dogs the deficiency may take two years or more to produce these osseous and subsequent nervous changes [376]. In cattle, cystic degeneration of the pituitary is reported [384]. Internal hydrocephalus and an increase in the pressure of the cerebrospinal fluid

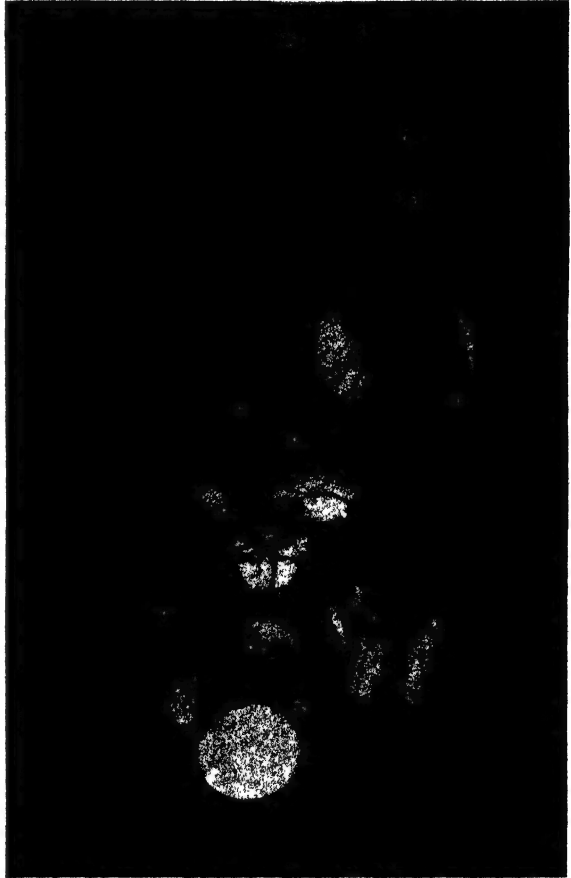


FIG. 8. Brain of young vitamin A deficient rat showing compression of the cerebellum into the foramen magnum and numerous herniations into the transverse sinuses.

also occur in dogs, but its cause is not clear. Against it being due to pressure from the osseous thickening are the observations of Moore and Sykes [286] that in calves papilloedema and the raised pressure decrease rapidly when vitamin A is given. This decrease could not, in the time, have been caused by a return to normal of the cranial bones. It seems most probable that the pressure of the cerebrospinal fluid is affected by lack of vitamin A because the ependyma is in origin epithelial and thus dependent, like all

other epithelia, on an adequate amount of vitamin A. The problem is further complicated by a report [850] that vitamin C also reduces the pressure of the cerebrospinal fluid of vitamin A deficient calves (p. 42).

Wolbach and Bessey [168, 323] also believe that the nervous system is compressed by the osseous, but the reason they give is that while lack

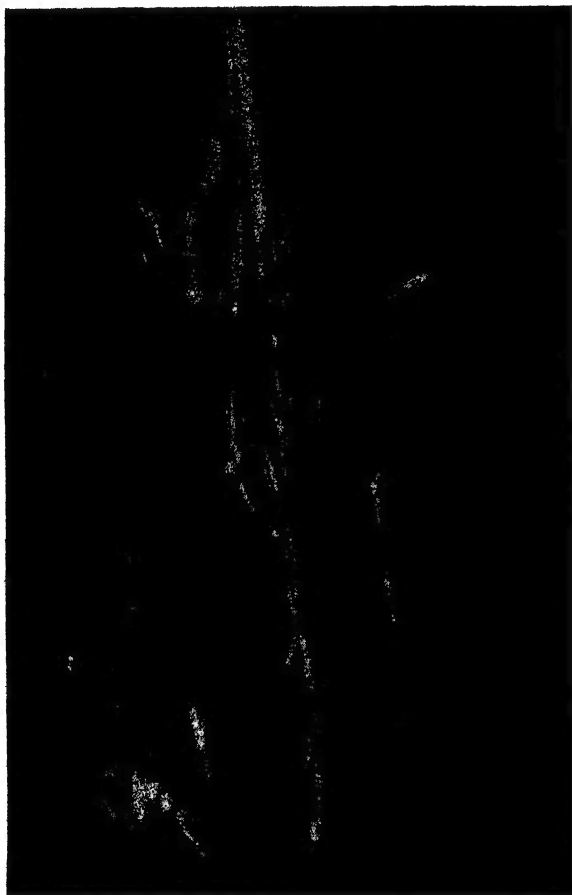


FIG. 9. Nervous system of young vitamin A deficient rat showing the disproportionate growth between the nerve roots and spinal canal, leading to herniations of the former. (The herniations have been dislodged so as to demonstrate them.)

of vitamin A stops osseous growth it does not affect that of the nervous system, so that the latter is compressed by its own relative overgrowth. Their extremely interesting papers, dealing chiefly with young rats, should be read in full. Their illustrations, two of which they have very kindly allowed us to reproduce, clearly show the effect of this compression on the central nervous system: the brain herniates into the venous sinuses and foramen magnum; the spinal nerves herniate into one or more of the intervertebral foramina which they pass before leaving the spinal canal; the spinal nerves are also kinked into large pits on the dorsal surfaces of the vertebral bodies, these pits apparently being due to the nerves eroding the bone as they are forced into the foramina of emis-

sary veins. The spinal nerves which are thus herniated show the classical histological picture of degeneration and regeneration following a crush injury. The fact that the vitamin A deficient axons regenerate is strong proof that lack of vitamin A does not directly affect the nervous system. The failure of some workers to find nervous degeneration in their deficient animals [184, 166] is explained by adult animals having been used, in which

no compression of the nervous system could have occurred except after very slow osseous changes such as take place in deficient adult dogs after two years or even longer [376].

Mellanby [178] some years ago advanced the theory that all the epithelial changes due to lack of vitamin A were really only secondary effects caused by the degeneration of the nerves stopping trophic impulses to the epithelia. This theory is now discarded: normal nerve fibres have been found in the deficient metaplastic bronchial mucosa, and a normal nervous system in animals with extensive epithelial changes [184, 166]; the return of the metaplastic epithelium to normal when vitamin A is given is more rapid than is consistent with nervous regeneration; xerophthalmia has been cured by carotene, but months later no regeneration of the trigeminal nerve has been found [172].

Vitamin A and the Osseous System. In rats on a vitamin A deficient diet the skeleton probably ceases to grow before any of the other tissues of the body, which suggests that vitamin A has a specific influence on the growth of bone [323]. The morphology of the bones of young rats, however, is not greatly altered in striking contrast to that of puppies, which has been described in the previous section. In calves there is stenosis of the optic foramina, causing papilloedema and compression and atrophy of the optic nerves [286] and cystic changes in the pituitary [384]. It would seem, therefore, that while vitamin A has a profound effect on the growth of bone, this effect varies in different species.

Vitamin A and Renal Function. The relationship of vitamin A to the kidney is not clear, but there appear to be four possibilities which are worth consideration: (a) The kidney merely acts as a storehouse for vitamin A. (b) The kidney destroys or excretes excess of vitamin A. (c) Vitamin A is necessary for the functioning of the kidney and, secondarily, reduces hypertension. (d) Impaired renal function allows vitamin A to leak away in the urine and also causes a toxic condition of the body which hinders it in utilizing vitamin A.

(a) The kidney normally stores small amounts of vitamin A which may rise considerably if the diet contains excessive amounts (p. 17), but it seems improbable that such an active organ as the kidney would be used merely as a storehouse especially when the liver already contains relatively huge amounts.

(b) However great the excess of vitamin A in the body no vitamin A ever appears in the urine unless the kidney is diseased (p. 20). Of course it is possible that the kidneys excrete the breakdown products of vitamin A (p. 20), but there is little proof that the kidneys destroy vitamin A themselves, as a first step in its elimination, except the observations of Belasco and Murlin [176], who found that renal tissue from rats on a high vitamin A intake had a slightly raised metabolism compared to that from control animals, from which they suggest an increased effort to destroy the surplus vitamin A.

(c) Our knowledge of the rather surprising effect vitamin A has on the excretory power of the kidney is chiefly due to Herrin [177], who found that in rats on a vitamin A deficient diet the urea clearance fell by twenty-

three to twenty-seven per cent., this being a purely functional effect, since not only was the urine normal, but histologically no structural changes were found in the kidneys. Further work on dogs, extended to include inulin clearance, confirmed the work on rats and also showed that excess of vitamin A raised the urea clearance above normal, though this could not be maintained indefinitely [178]. It was thought the effect was due to increased glomerular filtration, which has been confirmed by Bing [378]. Experiments on man were even more interesting [179]. When thirteen subjects were given 50,000 to 75,000 I.U. of vitamin A daily two showed no response, four had an increase in their urea clearance of from eleven to fifteen per cent., and seven had an increase of twenty-four to ninety-one per cent. The last group belonged to the type whose body weight fluctuates widely and rapidly. No subject showed any significant change in blood pressure or oxygen consumption. As in dogs the increased clearance could not be maintained, though in some cases it did not return to normal for 128 days. We have occasionally found vitamin A a powerful though transitory diuretic in daily doses of 72,000 I.U. by mouth in patients whose weight fluctuates rapidly. One child with nephrosis is reported to have been cured with vitamin A and unsaturated fatty acids [295].

Hypertension in some cases may apparently be reduced by vitamin A, though more work is required on the subject, especially as the apparent effects of vitamin A may be really due to the oil in which it is administered. Villaverde [356] reports that in one hundred cases of essential hypertension who were given 100,000 I.U. daily by injection or 200,000 I.U. by mouth for six weeks to several months, there was a decrease in blood pressure and relief of all symptoms in twenty-five cases, some improvement in fifty and no improvement in twenty-five. All these cases originally were found to have low levels of blood vitamin A and carotene. Taylor and others [380] could not confirm these findings in fifteen patients, but some were only treated for very short periods with smaller amounts of the vitamin. Dienst and Van Berber [357] on the other hand, found values in the blood at the upper limits of normal in hypertension and apoplexy, except when the latter had occurred recently, when values were low; they suggest vitamin A might be of value in apoplexy. In dogs [358] experimental hypertension caused by constricting the renal arteries has been decreased by the oral administration of 200,000 I.U. daily for three months followed by 400,000 I.U. for another three months: though the blood vitamin A rose to 8,000 I.U. per 100 c.c., there were no toxic symptoms. Katz and others [379] did not confirm these findings, and they also noted a further rise in blood pressure after dosing with the vitamin had ceased. In rats [359] a decrease in hypertension was caused by fish liver and fish body oils, but the significant fact was noticed that oxidation of the oils which destroyed their vitamin A increased their effect on hypertension.

(d) There are a large number of observations on man which show that chronic renal disease is associated with low stores of vitamin A in the liver (p. 18) as well as the reports of Long and Pyrah [128] and others [180] that renal calculi are frequently though not always [381] associated with poor dark adaptation, the most chronic cases tending to have the worst

adaptation [123]. It is not clear from these papers how much the renal function was damaged, but the most probable explanation of the observations appears to be that the damaged kidney drains away vitamin A (p. 20), or that the excretory power of the kidney being impaired, the resulting early "uræmia" may interfere with vitamin A, either in its absorption or more probably in its formation from carotene (p. 18), or even interfere with the formation of visual purple in the eye or cause a toxic amblyopia. In favour of this last view is the failure to cure the night blindness of nephritics with vitamin A [123, 180], and the statement by Clausen and McCoord [120] that in chronic nephritis vitamin A may be increased in the blood.

Vitamin A and the Endocrine System.

There is a large amount of conflicting evidence about the relationship of vitamin A to the endocrine system, nearly every statement made by one worker being contradicted by another. Here it is only possible to discuss recent research, referring the reader to the papers which are mentioned for a discussion on the validity of contrary findings.

The thyroid

among the endocrine glands has been most fully investigated; Drill [425] reviewed the literature in 1943. It now seems moderately certain that: (a) The thyroid stimulates the conversion of carotene to vitamin A, but does not increase the body's requirements of the latter. (b) Vitamin A decreases the effect of thyroxine in stimulating metabolism.

In man the importance of the thyroid for converting carotene to vitamin A is shown by the occurrence of night blindness in hypothyroidism [182], and by the low level of vitamin A (which is only slightly raised by

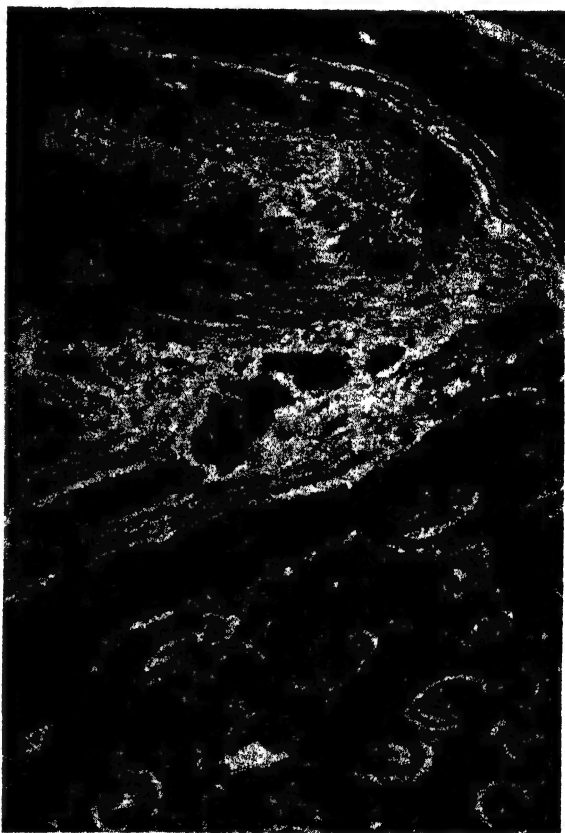


FIG. 10. Pelvis of kidney of an American infant filled with keratinized epithelium.

giving carotene) in the blood of cretins [183] and also by their susceptibility to respiratory infections. That over-action of the thyroid actually increases the conversion of carotene above the normal appears to be born out both by Moore [90] finding in the livers of patients dying from thyrotoxicosis larger stores of vitamin A than were present in any other human livers, and by normal cats (p. 18) being probably unable to convert carotene while thyrotoxic cats apparently can [184]. Further proof of the important rôle played by the thyroid in converting carotene to vitamin A is given by Fasold and Heidemann [185], who found that the milk of thyroidectomized goats was yellow with carotene but contained no vitamin A, in contrast to that of normal goats which contains no carotene but is rich in vitamin A. Thyroidectomized guinea-pigs are reported to be only able to store carotene and not vitamin A in their livers [186].

Destruction of vitamin A in the body is not increased by thyroxine, since besides Moore's findings in man mentioned above, he also showed that in rats given lethal amounts of vitamin A the addition of thyroxine did not have a protective effect by destroying the vitamin, which some workers have reported, but instead hastened the animals' death [187]; while Logaras and Drummond [188] found that the increased metabolism caused by thyroxine and dinitrophenol increased the storage of vitamin A in the liver.

The damping effect which vitamin A has on the activity of the thyroid was first suggested by McCarrison [189], who found that cod-liver oil delayed the metamorphosis of tadpoles, which has since been confirmed with purer vitamin A preparations. Experiments on animals by Logaras and Drummond [188] and many others [176, 184] have conclusively shown that vitamin A reduces the increased metabolism caused by thyroxine, while Belasco and Murlin [176] have found that the metabolism of thyroid tissue from animals taking large amounts of vitamin A is decreased compared to that of controls, this decrease being even greater if the animals have been given thyroxine as well. In fact vitamin A and thyroxine far from being antagonistic actually re-inforce each other in their action on the thyroid.

The clinical work on vitamin A and the thyroid is not satisfactory. The Mellanbys twenty years ago noted a clinical improvement in patients with exophthalmic goitre treated with cod-liver oil which they believed to be due to the iodine in the oil: some fifteen years later German workers claimed that vitamin A itself was of value in simple adolescent goitre [190] and in toxic goitres [191], but the vitamin A preparation "Vogan" which they used contains enough iodine to explain their results. We ourselves have occasionally but not constantly noted a marked clinical improvement after giving large doses of concentrated fish oils to patients with thyrotoxicosis who were already taking iodine, but we thought this was due to removing a mild dietetic deficiency.

All changes in the endocrine glands due to lack of vitamin A have been thought by some workers to be a secondary effect of a primary change in the pituitary. It is stated that the amount of thyrotropic hormone in the anterior pituitary is low in rats on a high vitamin A diet, and high in rats

on a deficient diet [192]. It has also been found that the factor in the anterior pituitary which stimulates the growth of the female genital system is increased in vitamin A deficient male rats, but Mason [180] has pointed out that this is a purely secondary effect due to the virtual castration of the male rats by the degeneration of the vitamin A deficient testes (p. 27), since deficient female rats showed no such changes in the pituitary's secretion.

The principle of the pituitary which stimulates lactation does not appear to be affected by vitamin A, since Williams and others [198] found that the amount of milk secreted by nursing mothers was not altered by varying their intake of vitamin A. Kepinov [194] in some very interesting experiments on starved frogs found that adrenaline did not accelerate the hydrolysis of liver glycogen to glucose unless vitamin A was previously given. Vitamin A apparently stimulates the glycogenic hormone of the pituitary, since it has no effect on frogs after the removal of the latter. That the function of the adrenals themselves may possibly be directly affected is suggested by Moore [88] and Popper [290] observing that they sometimes store large amounts of vitamin A. Wegelin [195] found that vitamin A checked the loss of glycogen from the liver which is caused by thyroxine, but here it seems most probable that the vitamin was directly decreasing the action of the thyroxine (p. 40) and not acting indirectly through the pituitary. The difficulty diabetics have in converting carotene to vitamin A (p. 51) is probably due to their impaired liver function and not to any pituitary effect. The changes brought about in the pancreas and thymus by a deficiency of vitamin A appear to be a direct effect on the glands themselves (p. 77). The cystic degeneration which occurs in the pituitary of vitamin A deficient cattle [884] is probably a secondary result of compression by the osseous hypertrophy which follows lack of vitamin A (p. 35).

Whether vitamin A in its action on œstrus and pregnancy acts directly on the ovaries or through the pituitary is not known. Mason and Ellison [56] observed that in rats œstrus is not stopped by a deficiency, but becomes delayed and irregular. Cannon [146] found that in severe deficiencies rats refused to mate, and with decreasing degrees of deficiency there was mating but no conception; conception but the foetuses were resorbed; death of the foetuses; prolonged gestation. Excessive amounts of carotene are reported to stop œstrus and a desire to mate [196]. (See also p. 27.)

Vitamin A and Fat Metabolism. For many years (885) an association has been noticed between a high level of carotene in the blood and lipæmia, such as occurs in myxœdema, lipoid nephrosis or diabetes, and more recently it has been shown that there is a definite correlation between carotinæmia and lipæmia [886]. Only lately, however, has the relationship between vitamin A and fat metabolism raised much interest though it has been thought for some years that vitamin A may be necessary for the transfer of fat across the gut wall in fish [271]; this apparently is not so in animals [848, 898]. But the converse, that fat (p. 15) and lecithin [861] aid the absorption of vitamin A, is satisfactorily proved.

In the only human case of hypervitaminosis A so far investigated there

was a lipæmia which disappeared when the excessive intake of vitamin A was stopped [886]. In children convalescent from pneumonia vitamin A raises the level of the serum lipoids and a high level of the latter increases the post-absorptive rise in the level of vitamin A in the serum [894]: the latter has, however, been denied [448]. Josephs [898] besides reporting his own well-controlled work on rats, has also reviewed the literature on the effect of vitamin A in raising the serum lipoids, the more important observations by himself and others being essentially the same in man and animals. Serum cholesterol varies in the same way as that of the serum lipoids and the raised levels of both following the administration of vitamin A ultimately fall even though vitamin A is still given. In previously deficient as compared to normal animals, the rise is greater and the fall slower; this probably also occurs in man which has lead Josephs to suggest that the level of the serum lipoids after giving vitamin A might be used clinically as a test for a deficiency of this vitamin.

In rats it is further reported that a deficiency of vitamin A has a specific effect in reducing serum lipoids and that in dogs, which normally excrete vitamin A in the urine (p. 21) and cannot be poisoned by vitamin A [886], there is no rise in serum lipoids after giving vitamin A.

In view of all the above, Josephs [898] believes that vitamin A has an important and specific effect on the metabolism of lipoids.

Relation of Vitamin A to other Vitamins. No relationship is known between vitamin A and the vitamin B complex or vitamin K. The connection between vitamin E and vitamin A is discussed on pp. 18 and 27, with the essential unsaturated fatty acids, or vitamin F on p. 19 and with vitamin D on p. 648.

The synthesis of vitamin C, by those animals which are able to synthesize it, is often said to be dependent on vitamin A; but the proof is meagre and unconvincing. In rats, Jonsson and his collaborators [849] state that deprivation of vitamin A causes a gradual disappearance of vitamin C from the blood, while at the same time changes occur in the incisor teeth which are said to be similar to those seen in the teeth of scorbutic guinea-pigs. Since lack of vitamin A affects the teeth of rats (p. 26) much more evidence than is presented would be required before these observations could be accepted. It is also stated that vitamin A deficient rats cannot utilize vitamin C given by mouth. Other workers [850] state that the urinary secretion of vitamin C in the rat is greatly reduced by lack of vitamin A, while Mitolo [851] reports that scurvy reduces the amount of vitamin A in the livers of guinea-pigs. Boyer and other [850] found that in calves the plasma vitamin C varied with the plasma vitamin A, especially when the latter was low, and the increased intracranial pressure resulting from lack of vitamin A (p. 35) was associated with diminished vitamin C in the cerebrospinal fluid. Giving vitamin C raised its level in the cerebrospinal fluid and, in three out of five calves, decreased the intracranial pressure. Rubin and Bird [852] could not confirm that lack of vitamin A prevents chickens synthesizing vitamin C.

THE PROVISION OF VITAMIN A IN HUMAN DIETS

Vitamin A and carotene are very far from being one and the same, but propaganda over food during war and the use in advertisements of such phrases as "provitamin-A" and "the amount of vitamin A (as carotene) in this preparation," have spread the erroneous belief that vitamin A and carotene are of equal value in the diet. Actually, unit for unit, more carotene than vitamin A is necessary if all requirements are to be satisfied, and also some stores of the vitamin are to be built up, since the body only utilizes carotene as efficiently as it does vitamin A when there is already a severe deficiency of both (p. 18). For banking vitamin A against any future shortage carotene is wastefully used compared to vitamin A. Also, if pp. 11 and 14 are read, it will be seen that while vitamin A is nearly always well absorbed, only five per cent. of the carotene in vegetables may be absorbed by healthy men according to one worker [80], and though this is probably too low a figure other factors, which do not greatly influence the absorption of vitamin A, may adversely influence that of carotene, such as poor general health of the body and of the bowel, the absence of fat and bile, and the taking of liquid paraffin either alone or in an emulsion. So it must be emphasized that if the tables on p. 46 are consulted about the carotene content of a food, the values given bear little relation to the amount of carotene which will be absorbed. Even after absorption hepatic and renal diseases, hypothyroidism, and diabetes may prevent the conversion of carotene to vitamin A (p. 18). All this means that where possible in health, and certainly in disease, some at least of the vitamin A requirements of the body should be supplied by vitamin A rather than carotene. This is especially important for children [309].

Vitamin A itself is found only in a very few foods, of which the commonest are butter, eggs, milk, honeycomb, liver, some fish and fish liver oils. Of these eggs, milk and butter are by far the most widely eaten, and for that reason it is important to realize that their vitamin content is not constant but depends entirely on the diet of the hens and cows. Broadly speaking, the more that eggs and milk are produced by "commercial methods" the less their value. This especially applies to eggs, which when produced on egg farms have only to conform to the naked eye appearance of eggs, since the fact that they are often too deficient in vitamins for any chick to hatch out of them does not here make the farmer feed the hens properly. The old belief that a dark yolk meant a good egg is undoubtedly correct, and explains why people still prefer the "farm yard" egg of their holidays to the pallid yolked egg of their town grocer. Sjollem and Douarth [201] have summed the matter up by saying "where poultry have access to pasture they eat enough grass to bring up the vitamin A content of the yolks to a maximum. This undoubtedly is often not the case with poultry kept to produce eggs for consumption." Of course the colour of the yolk is due to carotenoids, but these are a good indication of the content of vitamin A itself, except in the improbable event of the fowls having had very large amounts of cod liver oil [202, 395], when pallid yolks may be rich in vitamin A.

Cow's milk and butter are excellent sources of vitamin A, their content again being dependent on the diet of the cows. Watson and others [203] found that the vitamin A in milk could be doubled and the carotene trebled by altering the diet, though neither could be increased beyond a certain level which varied with the breed of cow. Oldfield [204] states that "one pint of milk from a pasture-fed cow may be equal in protective value to two pints from a stall-fed cow," and it has been suggested that in England, as in Finland, legislation should be passed to ensure that the diet of cows contains enough carotene to maintain the vitamin A content of their milk at a reasonable level [898]. The colour of milk and butter is not a good guide to the amount of vitamin A present, since vitamin A and carotene do not run parallel to each other in milk, but vary in their proportions with the breed of cow; Jerseys, for instance, have a yellow milk which contains nearly twice the carotene of that from Shorthorns, but only half the vitamin A [205]. Pasteurized milk, dried milk, etc. [899] have the same vitamin A and carotene content as fresh milk, even after storage for several months, but sweetened condensed skimmed milk has none. Goat's milk contains only vitamin A (p. 40) and so is quite colourless, which may in part explain the unreasonable bias against a valuable and sweet smelling food. Human milk should contain ample vitamin A and carotene for infants, but the amounts, of course, depend on the maternal diet, especially on its content of vitamin A itself (p. 50).

Margarine in England has now by law to contain as much vitamin A and twice as much vitamin D as "grass fed" butter: that is 550 I.U. of vitamin A and 60 I.U. of vitamin D per ounce [328]. Good butter may, however, contain nearly twice this amount of vitamin A [329]. Whether vitamin A is synonymous with carotene and vitamin D with irradiated ergosterol is left apparently to the margarine makers to decide, since the Ministry of Food in a personal communication refuses information on the subject. Butcher's dripping bought in the poorer parts of English towns in July had no vitamin A [207], though it had as much vitamin D as summer butter, and, of course, had a higher energy value than margarine.

Fish may contain considerable amounts of vitamin A in their body fat, so that fat fish like eels, halibut, herrings, lampreys, salmon and sardines are valuable, but Pyke and Wright [289] found no vitamin A in twelve brands of tinned salmon and in one "chilled" salmon, so that salmon as usually eaten in England is worthless from this point of view. In England also, Bacharach and others [396] have shown that the average individual daily intake of vitamin A from herrings either fresh, tinned, bloatered or kippered, is only 6 I.U.—a lamentable fact, as these fish are often cheap and plentiful: the herring fleet could provide three herring a fortnight for everyone [897]. Two fresh herring in the summer months, when they are richest in vitamins (p. 47), would provide roughly 500–1,700 I.U. of vitamin A, as well as having considerable antirachitic value (p. 654).

Fish liver oils may be amazingly rich in vitamin A, forming the only concentrated medicinal preparations in use. The total amount of vitamin A in fish and fish liver oils which is provided by the English fishing fleet

is 1,862 I.U. daily per person in Britain and this could be greatly increased [397].

Carotene and the provitamin A carotenoids are very widely distributed throughout vegetables and fruits. As a rough rule it may be said that carotene is always present in association with chlorophyll (the green colour of which masks the red of the carotene) and in yellow vegetables and fruits. Thin green leaves, like those of cabbage, spinach and lettuce, are especially rich in carotene, while the bleached stalks of celery and the white hearts of cauliflowers contain little or none; white flour and milled rice are again an example of the loss of valuable carotene with the loss of colour. The carotene content of tomatoes was not found to be altered when the plants were grown in eighty-seven different nutrient solutions, but growing or ripening tomatoes indoors reduced their carotene by over one-quarter [355]. Factors influencing the carotene content of plants, including the importance of boron, have been reviewed by Maynard [355.] The excellent paper by Graves [333] should be consulted for a discussion on the different biological values of carotene from different vegetables: carotene in red or yellow vegetables, like carrots, is very poorly utilized in comparison with that of green leafy vegetables, the latter being thrice as valuable—due possibly to their high content of vitamin E (p. 18).

In some tropical and sub-tropical countries like the Philippines, the Dutch East Indies, Ceylon, India, China, the West Indies and parts of East Africa the problem of child blindness due to lack of vitamin A is so widespread and so serious that Fitzgerald Moore [208] in a practical discussion of the whole problem concludes that the only hope of a solution is reinforcing with vitamin A concentrates the local vegetable oils and fats which are eaten by the inhabitants. Of these arachis oil is the commonest and forms an excellent vehicle for vitamin A. It must be stressed that it is no use introducing alien forms of vegetable fats rich in carotene, like red palm oil, to take the place of the local varieties, because both expense and custom will prevent their use.

Concentrated preparations of vitamin A are only available as some form of fish liver oil. The diets of children and adolescents, and even of adults, could be greatly improved by an increased consumption of ordinary unconcentrated cod liver oil or other fish liver oils, two teaspoons of which would roughly provide most of the daily requirements of vitamin A as well as other highly valuable nutrients (pp. 654, 785). Both in England [397] and South Africa [402] the fishing fleets bring back more fish liver oils than are at present consumed by their own countries.

The dilution of concentrated fish liver oils with arachis oil, to simulate cod liver oil, is unwise—though done in the “Ministry of Food Cod Liver Oil Compound with added vitamin D” which is issued free, if necessary, to pregnant women and children under five. The fat in this oil is less valuable and its vitamin A less stable than in genuine cod liver oil, nearly twice as much of the vitamin being destroyed by exposure of the oil to winter sunshine for eleven days [384].

Effects of Cookery, Storage, Canning, Freezing, Drying and Dehydration on Carotene and Vitamin A. Domestic cookery causes no appreciable loss

of either vitamin A or carotene, since neither are soluble in water nor easily destroyed by heat. The prolonged boiling of milk—though not rapid boiling or pasteurization—and the slow cooking of vegetables in stews is harmful, but there is little loss of vitamin A from butter during cooking and frying [209]. Most fats, however, which are used for frying—especially when they are reheated many times as in “deep fat frying”—develop an “anti-vitamin A” factor when heated which destroys part of the vitamin A activity of foods eaten at the same time [819, 820]. Probably such destruction is not of any practical importance except where foods such as commercial fish and chips are eaten in large amounts by the poor, who are always on the edge of a deficiency of vitamin A. The canning of fish probably destroys some vitamin A [896]. The cold storage of vegetables destroys small amounts of carotene [401], but canning, the ordinary methods of storing apples, oranges and tomatoes, and the domestic ways of preserving fresh vegetables and fruits, do not affect carotene, nor does drying peas and beans, though the slow sun-drying of fruit may be injurious. Dehydration of vegetables followed by reconstitution and cooking may cause a loss of from nil to seventy per cent. of carotene [854]; losses of course will vary greatly with the various processes, such as blanching, to which the vegetables may be submitted [400]. The commercial drying and evaporation of milk and its subsequent storage for a year was found not to reduce its vitamin A or carotene [210]. The “band drying” of eggs causes considerable loss of vitamins A and D, but “spray drying”—the method usually employed—has no injurious effect [444]. Human food is seldom sufficiently rancid to cause any serious loss of vitamin A [278].

AMOUNTS OF VITAMIN A AND CAROTENE IN FOODS

The following tables have been chiefly copied from those of Fixsen and Roscoe [267], which should be consulted if fuller figures about fish liver oils and vegetables are required. The figures for the vitamin A content of the flesh of fish are only approximate, since research has been chiefly directed to the amount of vitamin A in the body oils of fish. The amount of vitamin A in the edible part of fish has been calculated from the latter's fat content [268] on the assumption that this fat is the body oil referred to by research workers.

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
ANIMAL PRODUCTS			
<i>Beef</i>	60		
<i>Bone Marrow</i>	800
<i>Butcher's Dripping</i>	0		0
<i>Butter.</i> American	1,203-1,974	164-410	3,500
Danish. Nov.-Jan.	3,380-1,270
Feb.-Apr.	1,080-1,690
May-July	1,840-3,610
Sept.-Oct.	4,180-5,670

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
<i>Butter</i> , English	800-2,000
New Zealand	2,400
Scottish. Nov.-Jan.	2,410-1,140
Feb.-Apr.	1,220-1,530
May-July	2,560-2,950
Aug.-Oct.	2,990-2,200
Stall-fed cows	800-2,900
Goat	900
<i>Cheese</i> , Camembert	3,610
English Cheddar	5,500
Cottage	195- 729
Cream	2,110-2,320
Danish	2,400-3,500
Kraft. American	2,160
English	3,070
Swiss	1,970
Roquefort	2,500
<i>Eggs</i> , Duck. Whole	1,800	1,200	..
Yolk	2,900	1,800	..
Hen. Whole	700	600	..
Yolk	8,800
"	130-3,840	140-4,500	..
Dried	4,400
<i>Honeycomb</i>	4,096
<i>Liver</i>
Calf	52,600-159,800
Pig	12,000-36,700
Ox	12,700-41,800
Polar-Bear	1,300,000-
1,800,000
Seal (Arctic).	1,800,000
<i>Margarine</i> , Vitaminized	1,925	..	See p. 41.
<i>Milk</i> , Holstein. Summer.	1,510	550	..
Winter	1,010	430	..
Guernsey. Summer	850	1,700	..
Winter	510	780	..
<i>Irradiated</i>	Unchanged	Unchanged	..
<i>Dried</i> , Skimmed
Whole	428
<i>Pasteurized</i>	Like fresh
Goat	183-224	1	..
Human	200-500
"	125-750	15-60	..
" Colostrum	632
"	50-400
FISH
Bloaters	29
Carp	1,020
Eel	660-18,500
Herring
Canned, average	28
"	98-210
" with Tomato	53-105
Fresh, English
Mar.	88
Aug.	753
Sept.	193-238
Dec.	35-98

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY.
FISH—continued.			
Kippers	28		
Mullet	430-1,075		
Oysters	420		
Fresh salmon	4-120		
Canned salmon	0		
Chilled salmon	0		
Sardines	4,850-54,000		
Fish liver oils			
Cod (B.P., U.S.P.)	100,000		
Cod (Ministry of Health, p. 45)	100,000		
Cod (retail)	40,000-400,000		
Halibut	2,000,000-		
	36,000,000		
Tunny	512,000-8,000,000		
VEGETABLE PRODUCTS			
Cereals			
Maize	10-900	
Rice. Brown	34	
" Milled	0	
Wheat whole flour	102-456	
Wheat whole flour bleached	50-76% loss	
Wheat 85% extraction	50-300	
Semolina	291	
Spaghetti	52	
Vermicelli	0	
Fruits			
Apricot. Fresh	1,800-2,300	3,000
Sundried	5,100	
Banana	17	
Black currant	300-500
Blackberry	100
Date (preserved)	600	
Fig	80
Grape	15	
Guava	200
Orange juice	300-400	
" flesh	0-22	
Pear, English	80	
Palm fruit flesh	3,260	
Peppers (<i>Capsicum annum</i>) Green	110-1,080	
Red	3,390-37,700	
Pineapple. Fruit	60-160	
Tinned juice	50
Plum	0-230	
Tangerine	690	
Nuts and Oily Seeds			
Ground nuts	63	
Red palm oil		
African	110,000-306,000	
Burmese	44,000-56,900	
Malayan. Unripe	24,000-60,000	60,000
Ripe	66,000	190,000
Over-ripe	62,000	160,000

FOOD.	International Units of vitamin A or micrograms of Carotene or total vitamin A activity in 100 grams or roughly 3½ ounces.		
	VITAMIN A.	CAROTENE.	TOTAL ACTIVITY
<i>Vegetables</i>			
Beans. French	221-400	
Runner	600
Soya	450-900
Beetroot	0	
Broccoli leaves	12,000
Brussels sprouts	12,000
Cabbage	900
Carrot	2,000-9,600	
"	1,900
Cauliflower heads	38	
Cucumber	0	
Lentils	53-450	
Lettuce	1,500-2,400	3,000
Lucerne, dehydrated	3,000-6,000	
Marrow	0	
Onion	25	
Parsnip	80	
Pea	139	
Potato	28-56	
Radish.	3	
Soya bean	450-970	
Spinach. Fresh	2,630-6,500	
Tinned	12,280
Sweet potato. Brown	10	
White	0	
Tomato. Whole, green.	170	
" ripe	400	
" ripened indoors.	170-270	
Flesh	14,100-35,640	
Juice	320-590	
Tinned	4,280
Watercress	12,000

HUMAN REQUIREMENTS OF VITAMIN A AND CAROTENE

In Health. The latest and now widely accepted broad statement on the requirements of vitamin A was made by the Committee on Food and Nutrition of the U.S.A. National Research Council in 1941, which recommends a daily intake, as a mixture of vitamin A and carotene, of 5,000 I.U. for adults of both sexes whatever their occupation; of 6,000 I.U. for women in the latter half of pregnancy, and of 8,000 I.U. during lactation. During growth boys are stated to require 6,000 I.U. between the ages of sixteen and twenty, while girls of the same age need 5,000 I.U. Below the age of sixteen both sexes need 5,000 I.U., below the age of twelve 4,500 I.U., below the age of nine 3,500 I.U., below the age of seven 2,500 I.U., below the age of four 2,000 I.U., and below the age of one 1,500 I.U. These figures, as will appear from the following discussion, are parsimonious except for children, unless an unusually high proportion of the needs of vitamin A are supplied by the vitamin itself and not by carotene.

With a diet containing plenty of the other vitamins (p. 18) and with no liquid paraffin or paraffin emulsions used as aperients (p. 15), the requirements of vitamin A are only dependent on the weight of the body and not on age or physical activity (p. 21).

"The minimum (daily requirements of vitamin A or carotene) for significant storage, optimal dark adaptation and reproduction" are for vitamin A itself 60 I.U. per kilogram of body weight, and for carotene (provitamin A) 200 I.U.; that is for a man of 11 stone, or 70 kilograms, 4,200 I.U. and 14,000 I.U. respectively. For "normal growth, freedom from clinical symptoms, and little or no storage," 20 I.U. of vitamin A and 40 I.U. of carotene daily per kilogram are necessary. These figures are those of Guilbert, Howard and Hart [212], who in 1940 not only finally reviewed their own work, but also that of others on human requirements. They found a very close agreement about the correctness of these figures, which is further confirmed by Lehman and Rapaport's review [199], and also by Wagner [151]. The latter, from carefully controlled experiments on ten men, has stated that 2,000 I.U. of vitamin A or 4,000 I.U. of pure β -carotene are the daily minimum for preventing impaired dark adaptation. Lewis and Haig [213], from dark adaptation studies, originally put the daily minimum requirements of infants at 18–20 I.U. of vitamin A per kilogram, but this was later raised to 100–200 I.U. by Lewis and Bodansky [411], who based their observations on the level of vitamin A in the blood, though as this in infants has little significance (p. 57) the smaller amounts mentioned above, which are recommended by Guilbert, Howard and Hart to allow both for current needs and storage, should be aimed at.

It is important to build up reserves against any decrease in consumption such as tends to occur during the winter and during illness; a high intake has the further advantage of being a safeguard against any unrecognized condition of the body which may hinder absorption or the conversion of carotene into vitamin A (p. 18). Children especially need vitamin A itself, as they utilize carotene very badly [309]. There is also some evidence that there may be an inherited or familial need for unusually large amounts of vitamin A [199, 230]. The work of Batchelder [133] suggests the importance of ample supplies throughout life.

Pregnancy and lactation, which are a common cause of a deficiency of vitamin A in all countries (p. 63), increase the maternal need for vitamin A because the weight of the child both before and after childbirth should from this point of view be added to that of the mother; in fact her needs are roughly that of an 11-stone man, instead of a 10-stone woman. By taking a diet rich in vitamin A she not only reduces the risk of puerperal fever (p. 80), but also increases the stores of vitamin A in the liver of the foetus [99] and child, thus giving him the most important vitamin for good dental development (p. 26). It is important to give vitamin A rather than carotene during lactation, partly because the former appears to influence the vitamin A content of the milk more than the latter [81], and partly because it will be better absorbed and utilized by the infant. The milk of well-fed mothers contains ample vitamin A both for the infant's immediate needs and for increasing the low stores which are present at

birth (p. 17), providing about 3,000 I.U. as a mixture of carotene and vitamin A [81]. The maternal diet should be rich in fats, as there is experimental evidence that this aids the transfer of vitamin A to the foetus [99]. When breast feeding is impossible, cow's milk or dried full-cream milks give enough vitamin A, especially if the child is given cod-liver oil as well. Sweetened condensed skimmed milk, containing no vitamin A, is fortunately seldom bought in England, but in the tropics, where this waste product of civilization is widely sold to the natives, its consumption causes much widespread xerophthalmia and permanent blindness in children (p. 63).

The cost of foods rich in vitamin A and carotene means that at least half the populace of England are suffering from a mild vitamin A deficiency or are liable to develop it. To provide satisfactory diets which allow for storage is economically impossible, but at least the current needs of the body could be met by an increased consumption of stone ground bread and flour, cheap milk, cheap vegetables, and cheap fat fish like herring (p. 44). At present the price of the last three to the consumer bears little relation to the price paid to the producer.

For workers who have to use their eyes for matching colours it has been found that the provision of extra vitamin A lessened eye-strain and improved the health [214], so that it might even pay employers to provide free supplements of vitamin A to their workpeople. Clarkson [408] reports that in foundrymen the removal of particles of metal embedded in the cornea is made easier and fragmentation, flaking and splitting of the cornea while this is being done is prevented by the provision of extra vitamin A and, further, the men report their condition immediately instead of delaying over doing so.

General physical fitness and health in normally nourished people is not improved by an increased consumption of vitamin A. Bronsby and others [440] gave one half of 1,242 children between the ages of five and fourteen a capsule of arachis oil and the other half a capsule containing 4,000 I.U. of vitamin A and 600 I.U. of vitamin D. The capsules were given every school day for nine months. No effect was produced on growth, nutritional status, muscular strength, the teeth, the gums or the incidence of illness. In 214 men doing very heavy manual work, neither the weight, blood pressure, hæmoglobin, frequency of illness nor output were altered. Jenkins and Yudkin [489] gave 178 children, aged eleven to twelve, 5,000 I.U. of vitamin A and other vitamins every school day for a year and found no alteration in the pulse rate and vital capacity or in breath-holding and the 40 mm. endurance test.

In Disease. As a general rule it may be stated that most pathological conditions of the body interfere with the absorption of carotene from the bowel or its conversion to vitamin A in the liver, and also with the absorption and metabolism of vitamin A itself. Therefore in all acute and chronic illnesses the diet should provide vitamin A itself in larger amounts than those normally required, or fish-liver oil concentrates should be given.

Diabetes is the most important example of a chronic disease in which cartone cannot be converted to vitamin A, so that vitamin A itself must

be provided in the diet. In the early days of insulin, when the diet was extremely restricted, diabetics tried to satisfy their hunger with large quantities of green vegetables, with the result that xanthosis cutis or yellowness of the skin (p. 78) was common, because the body not being able to convert to vitamin A the large amount of carotene which was eaten deposited it in the skin. That this is the correct explanation is shown by Brazer and Curtis [126], who found that all of twenty young and middle-aged diabetics in America had poor dark adaptation, and nine had skin conditions suggestive of a vitamin A deficiency. Extra carotene only raised the already high carotene level in their blood still further without improving their dark adaptation, but extra vitamin A itself caused their dark adaptation to become normal. This improvement was only maintained as long as the vitamin A was given. Clausen and McCoord [120] also report that the blood of diabetic children in America contains too much carotene and too little vitamin A. Moore [90], however, observed that adult diabetic livers in England had large stores of vitamin A; this apparent contradiction is probably explained by a better provision being made in England than America for extra vitamin A rich foods to be given to diabetic hospital out-patients. The failure of diabetics to convert carotene to vitamin A, and also the difficulty they probably have in destroying carotene [386], appears to be due to their impaired hepatic function.

All diseases which interfere with the digestion of fat hinder the absorption of both carotene and vitamin A; carotene indeed is said not to be absorbed at all in coeliac disease [66]. The absorption of vitamin A itself is grossly decreased and delayed in coeliac disease [119, 211, 215], sprue [361], fibrosis and cystic disease of the pancreas [181, 215, 362], and congenital atresia of the bile ducts [94, 215]. It is possible that this poor absorption of vitamin A is increased by impaired motility of the gut [362, 370] and so can be improved by prostigmine or cascara [362]. Chesney and McCoord [211] found that in coeliac disease the vitamin A of the serum was low while the rise after a large dose of fish liver oil was relatively slight; it did not reach its low maximum for about nine hours, after which it fell very slowly, so that at the end of twenty-four hours it was still four times as high as the initial level. Normal children started with a much higher serum vitamin A, which rose after the oil to a maximum in about four hours and then fell, so that at the end of twenty-four hours the level was only fifty per cent. higher than at the beginning. This flat absorption curve for vitamin A in coeliac disease was found of use in differentiating it from chronic diarrhoea with a high faecal fat and a flat glucose tolerance curve.

Fever prevents the absorption of vitamin A and carotene and the liberation of the former from the liver into the blood. Heyman [66] states that toxic fevers reduce carotene absorption by a half, but he considers this largely a secondary effect due to impaired digestion of fats, while Clausen and McCoord [120] found the vitamin A of the blood low in fever, and considered that this was due partly to the diet, partly to the fever, since it also occurred when this was artificially produced. Even feverish colds halve the level of vitamin A in the blood [404]. Spector and his

collaborators [418] in an excellent review of their own work and that of others, agree that in acute and chronic infections the serum vitamin A is low and cannot be raised by giving vitamin A by mouth. They also found this in an infant with infected adenoids until the adenoids were removed, in children with uncompensated mitral stenosis (p. 18), and in children with eczema (p. 70), asthma and hay fever. May and others [215] point out that a low blood vitamin A in acute fevers does not mean low storage, since when the fever abates the level in the blood rises spontaneously without any vitamin A having been taken; this has been confirmed for typhus fever [407] and rheumatic fever [445] and in a very thorough study of children with pneumonia by Josephs [394]. The few cases of pneumonia [216] and measles [95, 98] on which a post mortem has been done after a large amount of vitamin A had been taken show in the former no extra storage and in the latter storage of only seven to ten per cent. of that taken. If all fever has the general effect of reducing the level of vitamin A in the blood, in

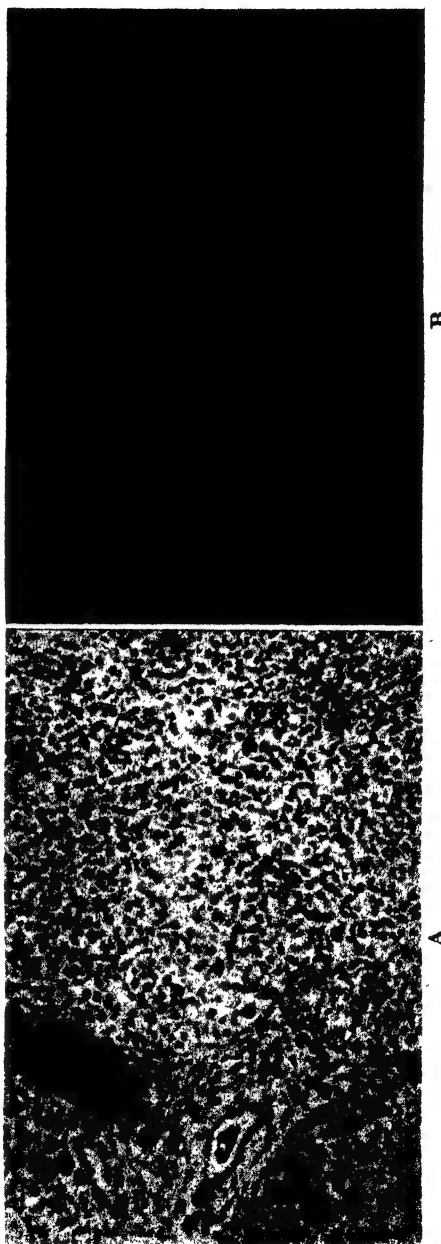


FIG. 11. Photomicrographs of a biopsy specimen from the liver of a patient with disease of the gallbladder with jaundice of short duration and without clinical signs of damage to the liver. A, routine histologic examination: extensive lymphocytic infiltration in the enlarged periportal field. No visible damage to the parenchyma. B, examination with fluorescence microscope: irregular vitamin A fluorescence is imparted almost only by Kupffer cells. The bile casts in the enlarged bile capillaries appear black. (See also Figs. 2 and 12.)

spite of adequate stores in the liver, it follows that the prevalence of

night blindness in consumptives [125, 406] may not mean that they actually are absorbing insufficient vitamin A, but that it is being stored in the liver and not circulated in the blood. Antidiphtheria injections are said to decrease the therapeutic effect of vitamin A given by mouth [304].

In hepatic disease the absorption of vitamin A is generally impaired [415], plasma levels tend to be low [412, 415, 416], even if hepatic stores remain high [290, 415, 416], and hepatic storage (p. 18) is generally decreased. The conversion of carotene to vitamin A is also impaired or does not occur [414]. According to Popper and his collaborators [415, 416] the low blood vitamin A in hepatic disease is partly due to the poor absorption and partly due to the inability of the diseased liver to transfer vitamin A to the blood. In support of the latter theory is the observation that there is a greater discrepancy between the amount of vitamin A in the blood and the

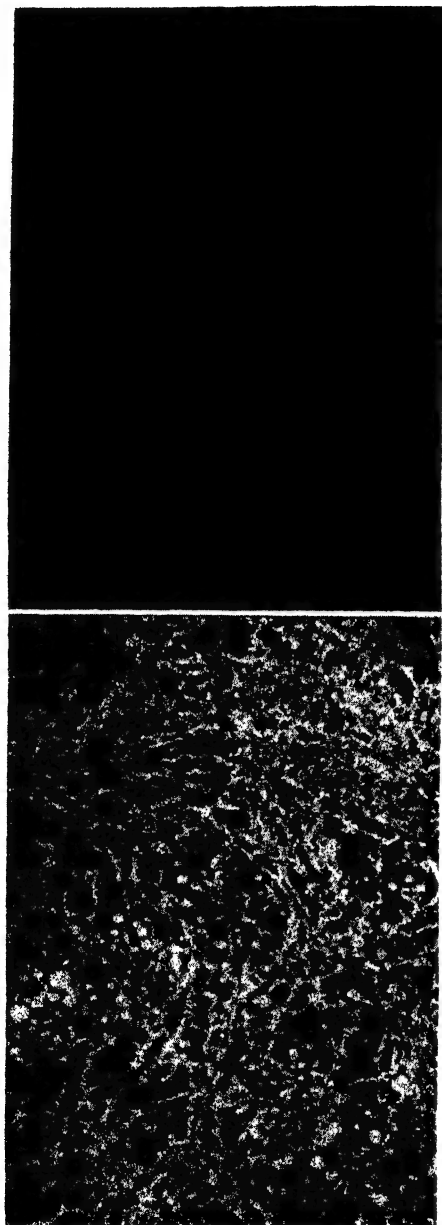


FIG. 12. Photomicrographs of a biopsy specimen from the liver of a patient with cirrhosis with jaundice. **A**, routine histologic examination : extensive proliferation of connective tissue with lymphocytic infiltration and proliferation in the bile ducts. Many fat droplets of various sizes in the hepatic parenchyma. The cytoplasm of the hepatic cells is dark. **B**, examination with fluorescence microscope : vitamin A fluorescence imparted by a few irregularly shaped Kupfer cells and by the large fat drop etc, the fluorescence of which is very low. (See also Figs. 2 and 11.)

liver when the fluorescence pattern of vitamin A in the liver (Figs. 11 and 12) is most disturbed [416]. The effect of fever, mentioned above, also

shows that there may be ample stores of vitamin A without these being mobilized for replenishing the vitamin in the blood.

Jaundice, especially when associated with cirrhosis or hepatitis [415], causes a low level of serum vitamin A [98, 412, 416] and hinders absorption, though the latter can be improved by bile salts [98, 418].

Liver function, it has been suggested, might be assessed by the effect of injections of carotene on the level of vitamin A in the blood, since a damaged liver would not convert the carotene to vitamin A and so the latter would not be increased in the blood. Höglér [414], injecting intramuscularly 6 mg. of carotene dissolved in arachis oil, found that in seven normal people the serum level of vitamin A rose by about 85 Lovibond units—from a resting level of 40–60 units—in 30 to 180 minutes, falling again in about five hours. There was no rise in six patients with hepatic cirrhosis and only a slight rise in five with catarrhal jaundice and two with cholecystitis complicated with hepatitis.

Parasites in the small intestine may adversely affect the metabolism of vitamin A, hookworm infections [304] decreasing the therapeutic response to the vitamin and lamblasis or giardiasis [405] impairing its absorption (see also p. 29).

If the figures given on pp. 17 and 18 for storage of vitamin A in the livers of adults and children are examined it will be seen that it is rather the chronic diseases and not the acute which have low reserves and so require special attention to their diets. This is especially true of chronic diseases which need dieting like ulcerative colitis (p. 16). The special problems raised by renal and thyroid disease and nystagmus have been discussed on pp. 37, 40 and 61. The use of vitamin A in the treatment or prophylaxis of infections of epithelial surfaces such as the "common cold," pneumonia, puerperal sepsis, typhoid fever, leprosy and tuberculous ulcers and senile vaginitis has been considered on pp. 28 to 32.

METHODS USED FOR RECOGNIZING HUMAN VITAMIN A DEFICIENCIES

Four methods are employed for determining whether a patient has a deficiency of vitamin A: Clinical examination, slit-lamp microscopy of the conjunctiva, estimation of vitamin A in the blood and measurement of dark adaptation. Though the last three of these are not generally feasible, being essentially methods used in academic medicine or in painstaking nutritional surveys, this does not matter in clinical medicine since xerophthalmia and keratomalacia, the only conditions urgently needing vitamin A, must be treated without waiting for scientific corroboration of the obvious clinical diagnosis.

Clinical Examination. The clinical signs of a deficiency of vitamin A are described on p. 68, so that all that need be said here is that a clinical diagnosis is only possible when the deficiency is already moderately severe, though some workers claim that a hesitant diagnosis can be confirmed by the finding of keratinized cells in the conjunctiva, respiratory tract and urine [111, 215].

Slit-Lamp Microscopy. Kruse (380) in 1941 aroused considerable interest by his claim that biomicroscopy of the conjunctiva of adolescents and adults revealed certain subepithelial opacities which were among the earliest detectable signs of a deficiency of vitamin A. Berliner [420], however, states that the opacities appear to have been only the common presenile or senile alterations which occur in the sub-epithelial layers of the conjunctiva, and the "spots" Kruse describes only the common pingueculæ. It is also most surprising that a lack of vitamin A should affect sub-epithelial but not epithelial tissues. Kodicek and Yudkin [367] agree with these main criticisms made by Berliner and they also report epithelial changes found by them while examining 496 school children by slit-lamp microscopy. These changes ranged from areas of superficial wrinkling with a transparent epithelium, usually placed midway between the limbus and external canthus, to areas of an opaque epithelium of a dead white or milky colour. The cause of these changes was not clear, and no claim is made that they were due to lack of vitamin A: they were less common among children between eight and ten than children of eleven, and more common in poor children and in boys than in girls; they were not related to the capacity for dark-adaptation, to weight and height, to the level of hæmoglobin or to intelligence. The exact significance and possible diagnostic value of these epithelial changes will be clearer when their response to vitamin A has been observed, but it would seem probable from their description, from their greater prevalence in boys (p. 22) and from their relation to poverty, that they are the forerunners of Bitot's spots (p. 72) and a sign of a deficiency of vitamin A.

The Level of vitamin A and Carotene in the Blood. In *adults* the normal values for vitamin A in the blood serum are probably between 100 and 300 I.U. per 100 c.c. In twenty-three subjects with normal dark adaptation Yudkin [381] found values varied from 72 I.U. to 157 I.U. per 100 c.c. with an average of 113 I.U. Values tended to be low in the early spring. Lindquist [217] states that below 70 I.U. dark adaptation is always impaired, and that only values above 110 I.U. can be definitely regarded as satisfactory. This is confirmed by Cowell [218], who found poor dark adaptation below 70 I.U. with normal values between 100 and 300 I.U., there being great individual variations which remained even after the giving of large amounts of vitamin A by mouth. Pett and La Page [219] state that dark adaptation improves as the vitamin A in the serum rises from 70 I.U. to 188 I.U. Kimble [220] gives the average in men as 127 I.U. and in women as 91 I.U., while other workers [422, 424] report averages for women of 146 and 96 I.U. From all these figures it appears fairly definite that values below 100 I.U. per 100 c.c. mean that the patient is deficient in vitamin A; though both the effect of fever on depressing the level of vitamin A in the blood while the stores are adequate in the liver (p. 52), and the rise in blood values which remains for over twenty-four hours after taking a large quantity of vitamin A (p. 52), mean that the significance of the level of vitamin A must be considered with these facts in mind. Several workers have also emphasized that moderately low levels may not mean low stores in the liver, but rather some abnormality in distribution [305,

806], while Yudkin [824] is so impressed by the different levels found in normal people that he considers blood estimations are valueless for diagnosing mild deficiencies; a level which is normal for one subject may mean a deficiency in another. Josephs [228] suggests that the vitamin A of the serum lipids gives a truer picture of the reserves of the body than does the vitamin A of the whole serum, since the former is not so affected by transitory absorption of vitamin A by the gut.

Pregnancy itself appears to have no specific affect on the level of vitamin A in the blood, apart from causing a slight fall due presumably to the increased requirements of the vitamin or to the dilution of the blood at this time. Thus Byrn and Eastman [422] found that in 157 white women at term the average level was 128 I.U. per 100 c.c., while in 25 non-pregnant white women the average level was 146 I.U.; in 187 black women at term the level was 112 I.U., and in 25 non-pregnant black women 128 I.U. Bodansky and others [421] report that in 70 women the average level after six months or less of pregnancy was 105 I.U., while the average for 62 women in the third trimester of pregnancy was 91 I.U. After pregnancy the levels tend to return rapidly to normal [422]. In 294 pregnancies, no relation was found between the level of vitamin A in the blood and puerperal infection (p. 80), premature labour, abortion, hæmorrhage or toxæmia; but in none of these cases was the level of the vitamin much below the normal [422].

At birth the average level of vitamin A in one series [424] of 143 infants has been reported as 49 I.U., values ranging from 24 to 79 I.U., while in another series [423] of 108 infants the average was 76 U.S.P. units, which fell to 37 at the end of the second day and rose again to 61 on the fourth day. In a third series [422] of fifty infants the average value was 91.8 I.U. There is no close relationship at birth between the levels of the mother and infant, at least when the maternal levels are not very low [422, 424], though the levels of the infant are generally lower than those of the mother [422, 424]; when the maternal levels are low the infant's may be higher [424]. Large supplements of vitamin A given during pregnancy or labour, though absorbed by the mother, do not affect the level of vitamin A in the infant [422, 424]. Toxæmias, obstetrical complications and premature labours, and twins are stated not to be related to the level of vitamin A in the infant [422], though smaller infants have a lower level [424] and the level in identical twins may differ greatly [424]. Lewis and others [423], after most careful work, consider that the low level during the first few days of life is due to rapid hepatic storage combined with an inability to release hepatic stores into the blood, so that relatively high blood levels of vitamin A can only be maintained—quite unnecessarily—by giving large amounts of vitamin A in the milk (see also pp. 11, 15).

Estimations of carotene in the blood are not of great clinical value, since carotene is probably useless to the body until it is converted to vitamin A. Thus high carotene levels may be present with a vitamin A deficiency, as in myxœdema, where conversion is impaired (p. 89), or may be very low in patients who are taking ample supplies of vitamin A

itself and little carotene. In fact carotene estimations are only of value in showing that carotene absorption is not impaired and, when vitamin A is estimated at the same time, in showing how efficient is the conversion of carotene to vitamin A and, possibly, in confirming a diagnosis of hepatic disease (p. 15). It must also be remembered that carotene absorption is dependent on many factors (p. 11) and carotene affects the blood content for as long as three days after being eaten [120], so that blood values have little meaning except when applied to a particular patient whose health and recent diet has been fully investigated. Thus Steininger and others [224] found that the carotene in the blood of thirty-four adults varied from 58 to 280 micrograms per 100 c.c., and Kimble [220] gives the average for men as 166 and for women as 187 micrograms per 100 c.c. Yudkin [331] reports values between 50 and 241 micrograms per 100 c.c. in normal subjects.

Early in pregnancy average values of 112 micrograms per 100 c.c. have been found, rising in the eighth month to 140 micrograms per 100 c.c. [421]. In new born infants average values of 20 and 28 micrograms per 100 c.c. have been reported [422, 424] with a range of 9 to 75 micrograms [424], the mother's carotene levels being on the average five to ten times that of their infants' [422, 424], and bearing a strong relation to them [424]. (See also p. 11.)

The methods employed for estimating vitamin A and carotene in the serum are based on the antimony trichloride colour reaction (p. 6) and the matching of the depth of the colour of the carotene against solutions of potassium dichromate or coloured glasses. The introduction of micro- and photo-colorimetric methods saves time and avoids the errors inherent in the comparing of colours by eye [220, 331, 372]. These estimations require a skilled worker and special apparatus, and so are not possible in ordinary medical practice.

Dark Adaptation. A slight decrease in the *final* degree of dark adaptation of which the fully dark adapted eye is capable and also, though not so constantly, a slight delay in the rate of early dark adaptation are generally considered to be among the earliest signs or symptoms of a deficiency of vitamin A, though the patient himself is seldom aware of these slight disabilities.

Measuring the extent and rapidity of dark adaptation has therefore been extensively used in nutritional research and surveys to unmask mild deficiencies of vitamin A, though it is an investigation which is not feasible in general practice since it requires both apparatus and experience. Either the Birch-Hirschfeld photometer or the Jeans-Zentmire biophotometer are generally used or the Hecht and Nagel adaptometer [151, 225] or the instruments described by Dow and Steven [325] or Yudkin and others [427]. The rotating hexagon, described by Livingstone [428] for rod scotometry, promises, when its uses have been more fully explored, to be of great value in investigating further aspects of the rôle of vitamin A in night vision.

The papers by Harris and Abbasy [108], Yudkin, Robertson and Yudkin [427] and Craik [429] should be read for discussions on what

Dark-Adaptation Curves and the Various Effects of Vitamin A on Dark-Adaptation

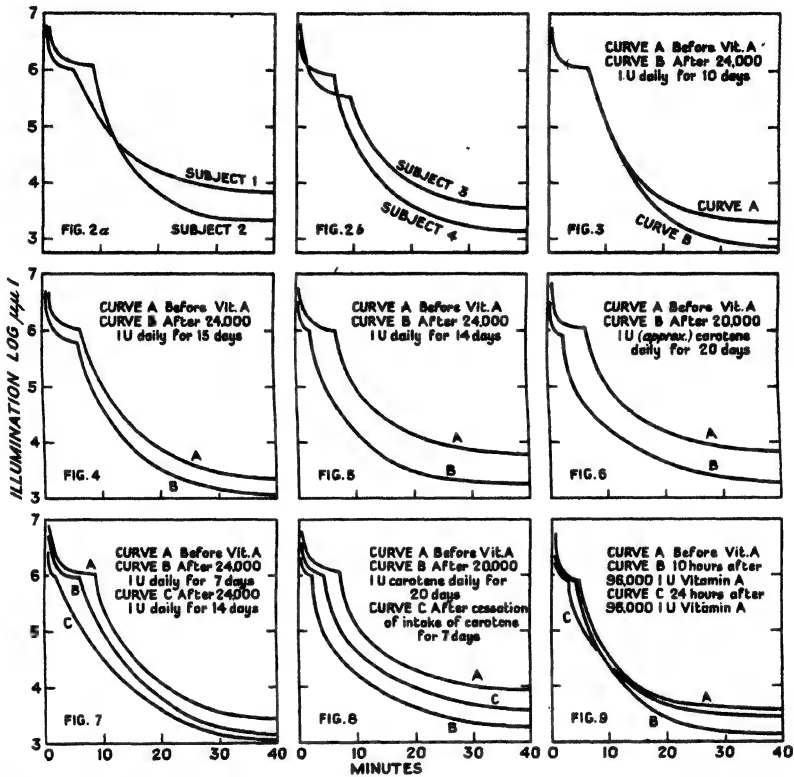


FIG. 13.

- Figs. 2a and 2b. Individual differences in curves of dark-adaptation.
 Fig. 3. Change in rod threshold only.
 Fig. 4. Change in cone threshold and rod threshold.
 Fig. 5. Change in rod threshold and transition time.
 Fig. 6. Change in cone threshold, rod threshold and transition time.

- Fig. 7. Gradual improvement with continued administration of vitamin A.
 Fig. 8. Improvement after administration of carotene; relapse after cessation.
 Fig. 9. Transitory changes produced by one large dose of vitamin A.
 Figs. 8-9 illustrate various effects of vitamin A on dark-adaptation.

instruments and what techniques give the most reliable results. The main differences in technique among different workers are the time the eyes are exposed to a preliminary bleaching of their visual purple (p. 38); the use of a fixation point during testing rather than allowing the eyes to move and so select their own most sensitive retinal area, thus avoiding any area which would give a fallacious impression of impaired adaptation due to congenital deficiency of the rods or localized pathological changes [428]; the distance of the eyes from the test object; the size and nature of the test object; and, most important of all, whether the whole curve of dark adaptation is plotted: this is discussed later.

The broad principles of testing dark adaptation are roughly the same in all instruments. The eyes are first exposed to a bright light to cause bleaching of the visual purple after which the light is extinguished and the patient is shown small illuminated test objects. As the eyes become more adapted to the dark the illumination of the test objects can be reduced without their becoming invisible. From the different degrees of illumination of the test objects and from the time taken for the test objects to become visible with these different illuminations, a curve of dark adaptation can be constructed (Fig. 13). Children under eleven or those who are mentally dull or deaf or whose vision is bad, are unfit for the test [108].

Early investigations on vitamin A deficiency were often done by measuring dark adaptation after the subjects had been only a few minutes or even seconds in the dark. But this measurement of the initial rate of adaptation may give completely fallacious results, since when the initial rate is rapid the final threshold or degree of adaptation may be low and it is the latter, not the former, which is *invariably* affected, if vision is affected at all, by lack of vitamin A [427]. The dark adaptation curves of two subjects may even cross as late as twenty minutes after the start of dark adaptation, so readings taken during the first twenty minutes of adaptation may give completely erroneous information about the relative state of the vitamin A nutrition of two subjects. The curves (Fig. 13) constructed by Yudkin, Robertson and Yudkin [427] clearly show the various types of curve which may be encountered, the different effects which vitamin A may have on the curves and the paramount importance, if only one reading is to be used for assessing dark adaptation, of taking the reading when dark adaptation is virtually *complete*. Other workers, including Dow and Steven [325], Hecht and Mandelbaum [235], and Basu and De [326] have also emphasized the importance of plotting the whole curve of dark adaptation and measuring its final extent. Complete proof that impaired dark adaptation is caused by lack of vitamin A, and not by any of the other conditions mentioned below, must rest on the final threshold being raised by vitamin A. Large amounts, such as 100,000 I.U. daily for ten days or longer, should be given, and blood vitamin A estimations also carried out to make sure that the vitamin is being absorbed and is circulating in the blood [307].

The validity of the dark adaptation test for showing mild degrees of a deficiency of vitamin A is now generally acknowledged. Thus Harris and

Abbasy [108] found that impaired dark adaptation was common in children whose diet was estimated to be low in vitamin A while it was rare in well nourished children. Steel [234] found that even when using a simplified test he obtained consistent readings which, when low, were improved by vitamin A. Both these important findings have been confirmed by Yudkin and others [427] who in a like manner reproduced curves on different occasions for the same subject with little variation, and improved adaptation with vitamin A. Cowell [218] reports that night blindness occurs when the level of vitamin A in the blood is below 70 I.U. rather than between his normal of 100 to 300 I.U., and in Pett and Le Page's patients [219] dark adaptation improved as the blood vitamin A rose from 76 to 133 I.U. in the four hours following the consumption of a vitamin A concentrate; others have noted an improvement within one to two hours [199] and ten hours [427]. Peek [230] and Josephs [805] have confirmed that there is a correlation between the level of vitamin A in the blood and dark adaptation. Hecht and Maudelbaum [235] found that while dark adaptation was often promptly impaired by a diet deficient in vitamin A, it took as long as six to twelve weeks for it to return to normal, in spite of large doses of vitamin A. This puzzling observation may possibly be explained by an instrument having been used which had a fixation point, which might mean that an area of the retina in which recovery from deprivation of vitamin was slow, or where the retina was damaged [874, 428], was being stimulated [427, 428]. Most workers who deny, like Caveness [292], the value of estimating dark adaptation have used a biophotometer, the fallacies of which have been pointed out by Harris and Abbasy [108].

Robertson and Yudkin [447] have shown that dark adaptation decreases with increasing age, this decrease being due to the smaller size of the pupil. Therefore in nutrition surveys based on dark adaptation studies allowances must be made for the age of the subjects.

Other changes which may be caused by lack of vitamin A are an alteration in the cone-rod transition time [235, 427] which is shown in Fig. 13 and alterations in the rod visual fields, which can be shown by rod scotometry [428].

Differential Diagnosis of Impaired Dark Adaptation. It must be remembered that other conditions apart from deprivation of vitamin A may impair dark adaptation, such as congenital night blindness, retinitis pigmentosa, or a detached retina [226]; starvation with a low blood sugar [227]; deficient oxygenation of the blood [227, 428]; lack of sleep, though not physical fatigue [231]; and also hysteria and possibly lack of other vitamins, both of which are discussed below.

Culpin [228] has pointed out that the nystagmus of coal miners is not in itself a grave drawback, but that miners often add to it functional disabilities, among which may be night blindness. Kellett [229] could find no definite proof that lack of vitamin A was a factor in causing nystagmus, and Campbell [801] has reviewed the literature on the subject. Soldiers who have to carry out dangerous duties in the dark may develop a purely functional night blindness. This was recognized in England in 1917, but

was not publicly reported at the time, because it was commoner in the German than English armies, leading to wastage of men in the former, where its cause was not understood [228]. In 1941 Wittkower and his collaborators [802] examined fifty-two soldiers complaining of night blindness. Most were found to have severe psychological disorders not, in origin, due to fear of fighting. The surprising and to us unjustifiable conclusion drawn from this examination is that "most cases of night blindness seen in this country are probably of psychological origin." It seems more probable that only the psychopath and malingerer spontaneously complain of night blindness: normal people are seldom worried by the condition. Harman [803] has discussed how to recognize the malingerer.

The importance of vitamins, other than vitamin A, for dark adaptation is uncertain, but the work of Harris and Abbasy [108], of Stewart [281], and of Wald and Steven [165] suggest that vitamin C is important. This is very definitely denied by Yudkin [824] after a careful study of six subjects with poor dark adaptation, one subject with severe night blindness and a deficiency of vitamin C, and five subjects with low normal adaptation. A paper by Kimble and Gordon [282] stresses as well the value of riboflavin. This latter paper has been frequently quoted apparently without having been read, because it is generally believed to state that riboflavin is in some cases necessary for the formation of visual purple in the eye itself. All that is really stated is that in a few patients who had both poor dark adaptation and a low blood vitamin A, large doses of vitamin A had no effect on either until riboflavin was given, when both became normal. In other words, riboflavin appeared necessary for the absorption or mobilization of vitamin A, but there is no proof that once there was an adequate supply of vitamin A in the blood the eye itself could not utilize it normally. Dr. Pollak has very kindly shown us convincing and well-controlled complete dark adaptation curves which leave little doubt that riboflavin alone does in some cases improve dark adaptation, though he has not yet investigated if this is due to a direct action on the retina or to an indirect action through raising the level of vitamin A in the blood. Morton [446] has reviewed the evidence in favour of riboflavin and not vitamin A being the chromophoric group of visual purple.

Having excluded all the above factors which, apart from lack of vitamin A, may cause poor dark adaptation, there still remain various physiological conditions which Phillips [283] has shown will produce individual differences in dark adaptation. A large pupil in the light retards dark adaptation, while a large pupil in the dark hastens it. Dark adaptation is always slow in old age irrespective of the size of the pupil, and, though general colouring has no effect, heavy pigmentation of the retina favours more rapid adaptation.

Alcohol and benzedrine may, according to Yudkin [824], improve dark adaptation, even when they cause no increase in the blood vitamin A. The improvement is transitory, is beyond that obtainable with vitamin A, and is apparently not related to the mental effect of the drugs. Clausen and others [824], however, report that alcohol does cause a rise in the level

of vitamin A in the blood, though this is generally slight ; in dogs, alcohol may quadruple the amount of vitamin A in the blood by the end of forty-eight hours [287]. Only a few negative observations have been made on the effect on dark adaptation of thyroid, dinitrophenol, caffeine, bromide, strychnine, amphetamine, phenobarbitone and morphia [324]. Adrenaline and stimulation of the greater splanchnic nerve raises the level of vitamin A in the blood of rabbits [324].

HUMAN DISEASES DUE TO A DEFICIENCY OF VITAMIN A

Probably half of the entire population of England and America take so little carotene or vitamin A that they either have definite symptoms of a deficiency, or are so close to a deficiency that they have no stores in their bodies to carry them through periods when vegetables, fish, and milk are costly, as in the winter, or when the amounts of these which can be eaten or bought are reduced by ill-health or unemployment. Figures based on what percentage of the population has poor dark adaptation or skin changes give too rosy a picture of their nutritional condition, as a recognizable deficiency only occurs when the body has already broken down from lack of the vitamin, this breakdown being preceded by a period when the functions of the body are at best being carried on under an extra strain.

Changes in the skin and poor dark adaptation are the earliest signs of a definite deficiency which can be diagnosed. Judging by poor dark adaptation, more than half the children from working-class homes in England were found to be deficient by Harris and Abbasy [108], while in America a similar or even worse percentage appears [199]. In some parts of the tropics a deficiency must be almost universal, since Fitzgerald Moore [208] reports that in the Philippines nearly one-third of the children attending a hospital had xerophthalmia, while in the Dutch East Indies, with a child population of half a million, nearly four thousand children between the ages of twenty-one months and fifteen years were blind, apparently from lack of vitamin A. Both in Africa and India skin changes due to lack of vitamin A were present in eighty per cent. of some groups of children [237, 238].

In English adults a deficiency is apparently less common than in children, Harris and Abbasy [108] finding only slightly impaired dark adaptation in half of thirty-eight working-class mothers, and in three of twenty-five middle-class males. But in America the figures appear to be higher, over one-third of one-hundred and sixty two medical students being found to be affected [199], and half the adults attending a hospital out-patient department [239]. Pregnancy is commonly associated with lack of vitamin A in England [218, 234], America [198], Holland [218]. Germany [240] and the tropics [222].

The effects of lack of vitamin A on (a) the skin, (b) the eyes, (c) the nervous system and (d) other tissues will be considered separately.

The Effect of Lack of vitamin A on the Skin. (*Toad Skin or Phrynoderma, Shark Skin, Keratosis Pilaris, Ichthyosis Follicularis, Lichen*

Pilaris, Lichen Spinulosus, Darier's Disease, etc.) The skin changes which occur as a result of lack of vitamin A were described by Nicholls [238] in India in 1933, who first used the name toad skin or phrynoderma, and by Lowenthal [236] in the same year in Africa, who made his observations without knowing that the importance of vitamin A for the skin had already been recognized two years before in China by Frazier and Hu [110]. In England a few cases were reported in children by Goodwin [127] in 1934 and by Pcmberston [127] in 1940 (five per cent. of a group of 3,000 children), while May and Wolff [242] in 1938 noticed changes in the skin and nails of an infant with xerophthalmia. In America Lehman and Rapaport [199] in 1940 reported nine cases in children and reviewed the

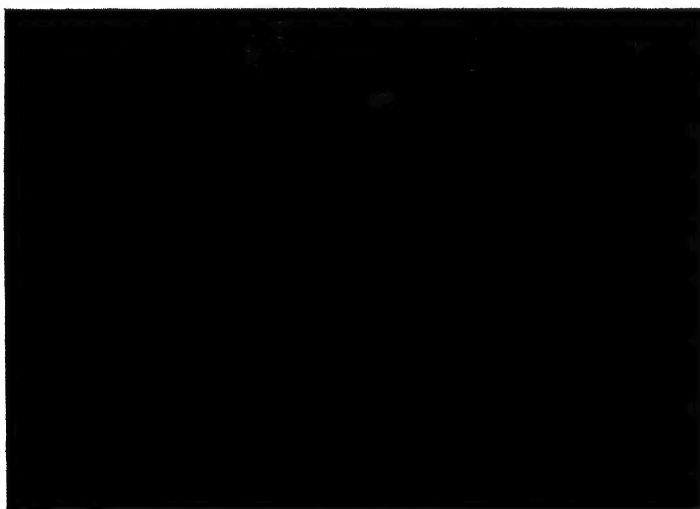


FIG. 11. Follicular hyperkeratosis on the extensor aspect of the arm of a girl aged fourteen. (Life Size. Great Britain, 1940.) Compare this with Fig. 15.

literature. Pallister [293] in 1941 found toad skin common in Malaya.

There is, however, still some uncertainty as to whether toad skin is due to a simple deficiency of vitamin A or whether some other factors are also involved. In favour of a deficiency of vitamin A being the only cause is the work of Lowenthal [236], who cured two of his cases with vitamin A alone, and practically all the rest with cod-liver oil, while Lehman and Rapaport [199] cured their cases with halibut liver oil. Pallister [293] in Malaya noted an association between toad skin and Bitot's spots. Steffens and others [248] produced typical changes in the skin of a man by a vitamin A deficient diet, and Nichols [238] and Lowenthal [236] have noted a close association between skin changes and night blindness or xerophthalmia, this association being apparently far commoner after than before adolescence.

Against these observations, however, must be put those of Aykroyd and Rajagopal [244] and Rao [248] who did not find any close correlation

between toad skin and xerophthalmia, or between the former and a diet deficient in vitamin A during a very extensive investigation of Indian schoolchildren. Frazier and Hu [110] and Sweet and K'Ang [111] found no correlation at all between the condition of the skin and eyes, so that they decided that in children the eyes but not the skin were affected by a deficiency of vitamin A, while after adolescence the skin chiefly suffered. This is confirmed by Frazier, Hu and Chu [110] who showed that in young children the skin is generally only xerotic and atrophic, follicular hyperkeratosis seldom occurring before adolescence (Fig. 20). The problem is still further complicated by the descriptions given by Fox [246] and Wiltshire [245] of the early skin changes in scurvy (p. 69) which appear to be almost identical with those of toad skin. The position appears to be that lack of vitamin A alone can cause toad skin, but that there is often some other factor which alters the reaction of the skin so that it is more sensitive to a deficiency of vitamin A. This ancillary factor may be either a second food deficiency [248], or the stage of sexual development [110], or a familial need for abnormally large amounts of vitamin A [127, 199, 220], or a racial susceptibility such as is apparently shown in India [238, 244] and Africa [237], but not in China [110, 111]. Whatever the cause the reaction of the skin varies so much that sometimes changes occur before there is any obvious involvement of the eye [110, 127, 289, 244] or even slight impairment of dark adaptation [248], while in other cases the eyes may be seriously damaged while the skin apparently remains normal.

The insidious onset of a dry rough skin, especially in those areas where the papular eruption occurs later, is the first cutaneous symptom of a deficiency of vitamin A. Such skins are not uncommon in children attending out-patient departments, though they are often missed, frank toad skin being rare at this age. Goodwin [127], Loewenthal [237], and Frazier and Hu [110] all stress this early symptom, which has been noted at all ages from infancy to old age and in both sexes. There is an increase in the spring [111, 119] after the deficient winter diet. The dry skin may be followed by a sudden local eruption which often spreads rapidly over the fronts and sides of the thighs, and the posterior and lateral sides of the forearms just below the elbows, and the fronts of the arms and shoulders. Some observers report that the eruption generally spares the front of the

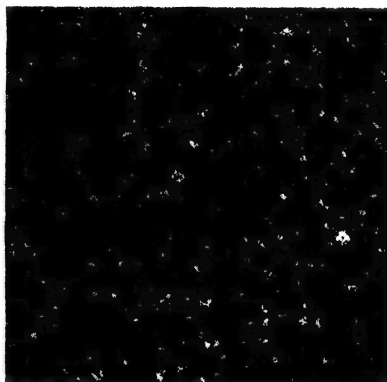


FIG. 15. Life-size skin print showing mild folliculosis on the front of the thigh of an English schoolgirl. Skin prints, in contrast to photographs, enable records of the condition of the skin to be made rapidly, easily and cheaply. Prints should be viewed with a reading-glass giving five magnifications. This figure does not do justice to the technique first described by Dr. Hugh S. Stannus in *The Lancet*, 1944, ii, 359.

chest [238], the groins and axillæ, and the backs of the hands and feet [110], but others state that it may ultimately cover the whole body apart from the face, which is seldom involved [238, 248] though "black-heads" are common [110, 111]. The scalp is not affected, but the hair may be dry and brittle and the nails may have transverse or longitudinal ridges [111],

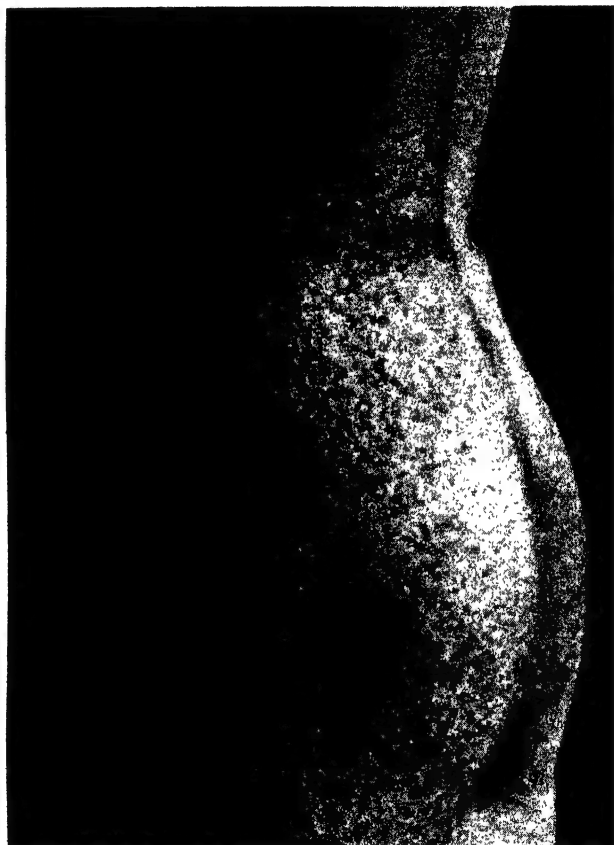


FIG. 16. Diffuse involvement of hair follicles due to vitamin A deficiency in a fourteen-year-old Chinese boy: his skin is also shown in Figs 17 and 18. Hyperpigmentation of the conjunctiva was the only ocular sign of vitamin A deficiency.

though generally the hair and nails are normal [110]. Increased pigmentation [111] both of the papules and the skin, which has been likened to argyrosis by Mu and others [247], is sometimes seen in coloured patients, being analogous to the scleral pigmentation (p. 78). Itching has been reported to be present [236, 238] and absent [248].

The eruption consists of dry horny round or oval sharply defined papules, varying in diameter from that of a pin's head to as much as a

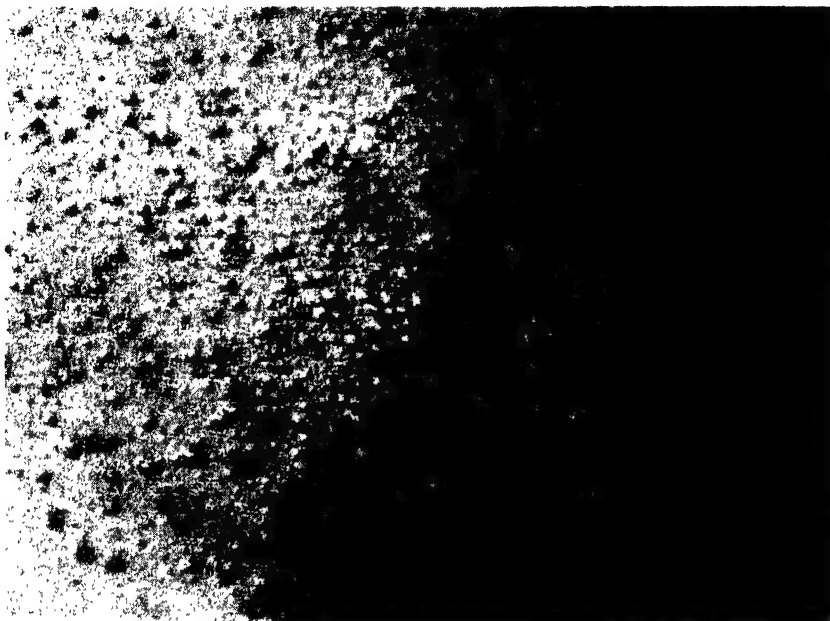


FIG. 17. Follicular hyperkeratosis, with projecting horny spines, before treatment. (See also Figs. 16 and 18.)



FIG. 18. The same case shown in Figs. 16 and 17 after five weeks of treatment with a vitamin A concentrate and cod liver oil, which provided about 68,000 I.U. daily.

quarter of an inch [286]. The size of the papules increases with the duration of the deficiency, in the early stages being more easily felt with the fingers than seen, while later the skin looks from a distance as if many split lentils had been stuck upon it. Each papule is formed by hyperkeratosis of the pilosebaceous follicles, and has a hard keratinous core which can be picked out, leaving a small pit. Often broken or coiled up unerupted hairs are found either projecting through the papule or imprisoned beneath. The papules seldom, if ever, undergo pustulation [111, 286, 288, 248], though Young [294] believes that the skin is more susceptible to fungus infections. Rao [248] and Frazier and others [110] have

reported skin changes in keratomalacia which are typical histologically of phrynoderma apart from the papules (Fig. 20).

Microscopical examination of the skin in phrynoderma shows that the papules are composed of masses of keratinized cells which have been shed from the pilosebaceous follicles, becoming compressed in their centres into horny amorphous plugs [127, 286, 248]. These block the hair follicles and the sebaceous glands which then tend to atrophy. There is also hyperkeratinization of the epidermis, especially round the papules, and a thickening of the

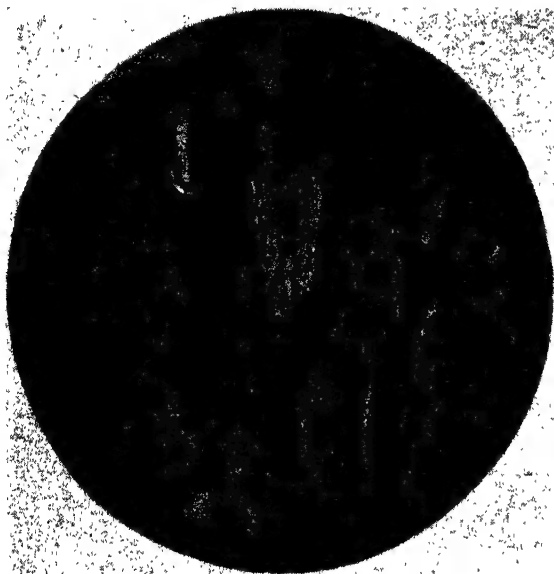


FIG. 19. Follicular hyperkeratosis in a boy aged fourteen. (Great Britain, 1940.) Note the plugging of the hair follicle with keratinized material; the absence of the sebaceous gland; the increased keratinization of the superficial layers of the skin; and the infiltration of small round cells near the base of the follicle.

stratum corneum and sometimes an increase in the pigment cells and a mild lymphocytic infiltration near the base of the follicle. The sweat glands are not markedly changed, but do not appear to be secreting, and they are often plugged with keratinous material. Vitamin A, in spite of its importance for the skin, is not present in the epidermis [290].

The differential diagnosis is from acne and from early scurvy. In acne the skin is greasy instead of dry, the eruption is mostly limited to the face and front and back of the chest, unlike that of toad skin, and it mainly occurs during adolescence and early adult life. Pustulation of the papules is the rule rather than the rare exception, generally leaving behind small scars which are never seen when uncomplicated cases of toad skin are cured.

The earliest sign of scurvy, according to Wiltshire [245] and Fox [246], is a skin eruption which is identical with that caused by lack of vitamin A, since the skin is dry and the papules are formed in the mouths of the pilosebaceous follicles by masses of keratinized epithelium in which or under which are broken or coiled up hairs. But these papules appear to be rubbed off easily and leave behind them pink follicles which have not been described in toad skin, and also their distribution is slightly different, being more confined to the legs. The perifollicular hæmorrhages which would confirm a diagnosis of scurvy only appear some weeks after the papules.

The long list of names at the head of this section probably were all



FIG. 20. Showing the atrophic condition of the epidermis of a Chinese child approximately three years of age who had generalized xerosis of the skin but no obvious follicular keratosis. There was pronounced xerosis of the conjunctiva, which was wrinkled and leathery, and early keratomalacia. The child had had diarrhoea for a month. The section is from the abdominal wall.

coined to describe the eruption due to lack of vitamin A before their common origin was recognized [127, 199].

Chronic ulcers of the skin are a very rare complication of phrynoderma, and when they do occur only heal with vitamin A [111, 249].

Treatment with vitamin A is entirely successful. The first sign of recovery is a return of sweating so that the skin within two or three weeks no longer feels dry [110, 199], though it does not return to normal for two to nine months, the shorter period being on very high doses of vitamin A, such as 100,000 I.U. a day. Concentrated preparations or even injections should be used when diarrhoea is present [111]. The keratotic plugs in the follicles are reported to be extruded as tiny rice-like bodies, but by remaining partly adherent to the skin they give to it a shaggy appearance

[199]. Ultimately the epidermis and the follicles return to normal and new hairs develop.

Darier's Disease. Since Peck and his collaborators [480] in 1941 suggested that Darier's disease was due to a deficiency of vitamin A, about a dozen further papers on the subject have appeared in *The Archives of Dermatology and Syphilology*, of which the most important are a further paper by Peck and others [480], and a paper on English cases by Carleton and Steven [482]. Darier's disease was also discussed at a meeting of the Royal Society of Medicine [483] in 1944. It now seems that vitamin A in large doses by mouth will greatly improve or even cure the condition in some cases, though in other cases treatment has no effect whatsoever. Excessive oral doses of vitamin A—300,000 I.U. daily—may cause the papules to become bullous, and massive injections of vitamin A are said to reduce the level in the blood [481], an unexpected finding which is discussed on p. 14. The suggestion that it is only one of the two genetic types of the disease which responds to treatment is disproved by Carleton and Steven [482] who cured a mother but not her son and a brother but not his sister. The level of vitamin A in the blood is just within normal limits [482, 487] or definitely low [480] and is raised and maintained in most cases only by continuous and large doses of vitamin A [430]. Dark adaptation may be normal [482], or impaired [430].

Ichthyosis. Rapaport and others [484] in 1942 reported six cases of ichthyosis all of which were very considerably improved by vitamin A, and also gave references to similar results obtained by two other workers. The vitamin was given orally in daily doses of 60,000 to 200,000 I.U. or intramuscularly two or three times a week in doses of 100,000 I.U. Some cases responded only to the former method of administration and some only to the latter. Improvement occurred within a few weeks, but relapses, especially in the winter, were common if the treatment was stopped. All the five cases who were tested had poor dark adaptation and in five cases there was a family history of ichthyosis.

Eczema. The absorption of vitamin A in infantile eczema is said to be impaired [485] and the level in the blood low [418]. Gross [486] reports curing eighteen of twenty-four cases of nummular eczema with 75,000 U.S.P. units daily. (See also vitamin F.)

Other Skin Conditions. Many other skin conditions have been treated by vitamin A, but the review by Carleton and Steven [482] and the investigations by Cornbleet and others [487] on the level of vitamin A in the blood of patients with thirty-two different skin conditions, leaves little doubt that, with the possible exception of *pityriasis rubra pilaris* [487], vitamin A will benefit no conditions other than those already discussed.

The Effect of Lack of vitamin A on the Eyes. The earliest detectable result of a lack of vitamin A on the eyes is a slight impairment of dark adaptation, the underlying physiology of which has been described on p. 32, and the methods for its detection and its differential diagnosis on p. 58, so that here it is only necessary to point out that the condition is seldom noticed, especially by those living in well-lit towns, until it has

become very pronounced. Few patients with mild night blindness are sufficiently observant to realize that their twilight vision is better in the early morning than in the evening, though this valuable diagnostic point was noticed years ago in badly fed slaves [226]. Sometimes, however, as in poorly fed Newfoundland fishermen [1], night blindness comes on suddenly after a long day in very bright sunlight, so that there is complete blindness in the evening dusk though normal people are seeing perfectly. Sudden changes in diet, such as that of Orthodox Russians during the Lenten fast, may also precipitate frank night blindness [250].

The first change in the eye which can be observed clinically is a drying or xerosis of the eye, known as xerophthalmia, which is generally accompanied by photophobia. In early cases the condition may be unmasked by holding the eye open for a minute or two when the lustre is lost through the rapid drying of the eye. This is due to the metaplasia of the conjunctival epithelium stopping the secretion of the mucous cells, which can be confirmed by finding keratinous cells in gentle scrapings of the eye [111]. Wrinkling of the conjunctiva can also be seen at an early stage. Later the conjunctiva may become thick and leathery from the gross keratinization. (The diagnostic value of slit-lamp examinations of the conjunctiva is discussed on p. 56.)

Once xerosis is present no further changes may occur, or the whole eye may be rapidly destroyed. The patient suddenly complains of a feeling of a grain of sand in the eye, which is followed by photophobia, lacrymation, inflammation, and a sticky discharge. These symptoms are due to the dry thickened conjunctiva wrinkling up, which gives the sensation of a foreign body in the eye, while the discharge is caused by a secondary infection [111]. The condition is now serious, since the stroma of the cornea may become oedematous, necrotic, and weak, so that

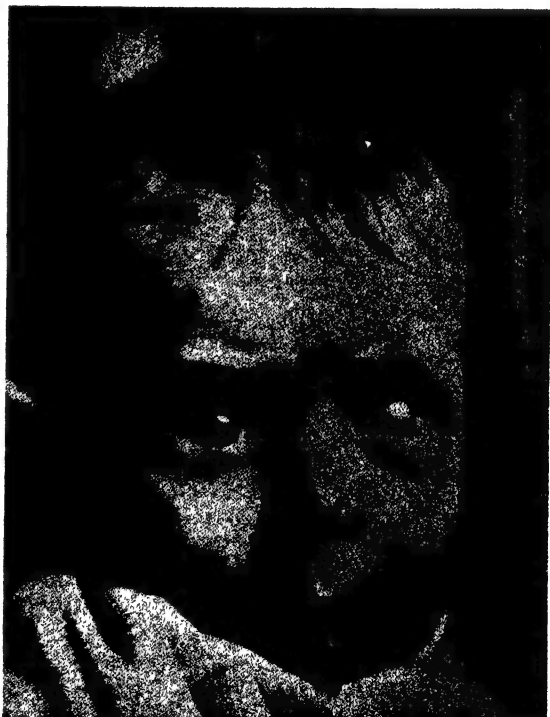


FIG. 21. Keratomalacia in a Danish infant (see p. 2). The disease arose after two months' feeding with oatmeal. After treatment with cod-liver oil the right eye improved and became almost normal. The left cornea is largely necrosed.

keratomalacia occurs. The first sign is a spreading opaque white spot in the cornea which can grow so rapidly that the sight may be lost in a few hours. At the same time secondary infection may lead to ulceration and finally perforation of the weakened cornea, with destruction of the eye. This fulminating course is commonest in infants [280], even having been present in one of Maxwell's [148] at birth, but it can occur at any age [418].

One-quarter of Sweet and K'Ang's cases [111] and of Blegvad's [280] had one eye involved for several weeks before the other.

The vascular changes which occur in the cornea and their possible relation to a local secondary deficiency of riboflavin are discussed on p. 25.

De Haas and Meulemans [222], investigating vitamin A in the blood of children suffering from xerophthalmia, found none in six and 4, 6 and 8 I.U. per 100 c.c. in three and 22 I.U. per 100 c.c. in one with perforation of the cornea.

Recovery, apart from scarring of the cornea, is always possible if perforation has not taken place. The treatment of acute cases in infancy is to give immediately 20,000 I.U. of vitamin A subcutaneously followed by cod-liver oil daily [222]. To older patients much larger doses should be given by injection [288]. Treating the eye itself with cod-liver oil would appear to be unnecessary, since Røth [251] found that in rats with xerophthalmia cod-liver oil placed only in one eye cured the other just as quickly, while the general condition of the animals improved, so that the effect of vitamin A was not local but general through its absorption into the body. In trauma of the eye cod-liver oil and liquid paraffin were equally effective, while Lichenstein [252] reports that the bactericidal properties of cod-liver oil are not due to its vitamins but to other properties. In fact the local treatment of the eyes should be that generally used for inflammatory conditions. Follicular conjunctivitis in children has been stated to be due to lack of vitamin A and to respond rapidly to 18,000 I.U. daily [304].

In infants [280] bronchopneumonia, due to infection of the metaplastic epithelium of the respiratory tract, is the usual cause of death. In adults [418] gastro-intestinal disorders, being both a cause and consequence of lack of vitamin A, often precede or follow xerophthalmia.

Prolonged mild deficiencies of vitamin A lead to the formation of "Bitot's" spots which were first described by Bitot [253] in 1863 in patients in the foundling hospital in Bordeaux: "Un assemblage de points d'un blanc éclatant, produisant comme une tache nacréée, ou argentées à côté de la cornée transparente." The "taches" fluctuated in size with the degree of night blindness, being larger when it was most severe. They were always placed just lateral to the cornea on the equator of the eye, generally being in the shape of a triangle whose base was slightly concave and about 5 mm. in width, while the sides of the triangle were about 8 mm. Sometimes, however, they were round or oval, and might also be composed of fine lines as well as dots. Very occasionally a few scattered dots were seen to the inner side of the cornea. Bitot also pointed

out that the "taches" were a definite change in the conjunctival epithelium itself, which might have either a rough or striated surface. Nicholls and Nimalasuriya [254] from extensive observations on several hundred cases in children in Ceylon have confirmed this picture, though night blindness was not usually present.

They state that the first changes are a slight thickening and pigmentation of the scleral conjunctiva, which is followed by a heaping



FIG. 22. Bitot's spots in a Singhalese child. The left eye shows thickening, pigmentation, and white striated patches of the temporal bulbar conjunctiva. This was also present in the right eye. The nasal bulbar conjunctiva was free from these changes.

up of epithelial cells which stand out white against the pigmented background, and are generally striated, looking like "a dab of chalk paste striated with a pin." They are only seen on the inner side of the cornea in two per cent. of cases; they never ulcerate; never occur over the cornea itself, though generally approaching to within 1 or 2 mm. of its edge; and mostly appear in only one eye. May and Wolfe [242] reported that in their English infant the triangular Bitot's spots were on each side of the cornea and appeared to be covered with foam, and Aykroyd and Rajagopal [244] describe the Bitot's spots in their Indian children as yellowish foaming patches. Nicholls [254] suggests that this foamy appearance is due to a very rapid and loose piling up of epithelial cells which does not occur in communities where a chronic deficiency of vitamin A for generations has led to a more chronic and slow reaction to the deficiency. The increased general pigmentation of the sclera—also noted by Mu [247]—probably only occurs in dark races, and its diagnostic value before Bitot's spots appear is still uncertain. Early conjunctival changes only visible with a slit-lamp are discussed on p. 56.

Treatment is with large doses of cod-liver oil or vitamin A concentrates, recovery being slow [244, 254], as it is in phrynoderma.

Asthenopia is stated to be often caused by lack of vitamin A, patients continuing, in spite of the usual treatments, to complain of a dislike of bright lights, headaches and difficulty in seeing while driving or at theatres, or fatigue whenever they use their eyes. Impaired dark adaptation is present. Cordes and Harrington [255] gave 30,000 I.U. of carotene daily for a month with complete relief of all symptoms in seventy-nine per cent. of eighty-two cases between the ages of thirteen and seventy-three, though as some of their patients became yellow during the treatment vitamin A should be used if such large doses are really necessary. Similar results have been reported by Vanzant [300].

The Effect of Lack of vitamin A on the Nervous System : Lathyrism. The experimental work discussed on p. 84 shows that deprivation of

vitamin A causes degeneration of the nervous system in animals, though the degeneration is surprisingly severe before there are any clinical symptoms. In man no neurological symptoms are generally associated with those conditions, such as keratomalacia, where they would be expected if the central nervous system were affected by lack of vitamin A. There is, however, the possibility that in man, as in animals, the degeneration gives such tardy symptoms that they seldom occur before the deficiency has been remedied or death occurs, unless the degeneration is accompanied by a second deficiency (p. 84). This is supported by Nicholls [256], who in Ceylon constantly found degeneration of the spinal cords of children dying with symptoms of a vitamin A deficiency, though he also found it in two children who had not been clinically deficient. He also points out that pregnant women in Ceylon often have transitory neurological disturbances which he is inclined to attribute to lack of vitamin A, since this is very common while beri-beri is rare. He previously reported [238] that prisoners with phrynoderma and eye signs generally had neuritis and diarrhoea; the latter condition may have caused or increased a deficiency of vitamin B, or both may have been further symptoms of lack of vitamin A (p. 77). In the same way as phrynoderma may require both lack of vitamin A and some other factor for its development (p. 65), Mellanby [171], from his work on puppies, has suggested that vitamin A is necessary for the health of the nervous system in man, its lack, however, only becoming important when a second deficiency or some toxin is present: so that beri-beri is accentuated by lack of vitamin A and gangrene in ergotism occurs alone when vitamin A is plentiful, but when it is not convulsions also appear, because the nervous system needs vitamin A to protect it against the ergot. Mellanby also suggested that lathyrism is a disease not only due to toxins which are always present in the germ of grain but also to lack of vitamin A, since puppies on a high cereal vitamin A deficient diet developed a more serious degeneration of the cord when they were also given rye germ and, in two cases, wheat germ or dried beans.

Lathyrism is a disease which in the past has been ascribed to the eating of a vetch of the *Lathyrus* family during periods of famine, either deliberately in areas where it is used as cattle food, or by mistake where it has grown among the corn. But the published accounts of the disease give such varying symptoms, and in several instances so definitely rule out the possibility of any kind of *Lathyrus* being the cause, that it appears certain that lathyrism is not one disease but a group of rather similar diseases, only sharing in common a background of famine and some form of paralysis of the legs.

The only cause common to all outbreaks of lathyrism is a deficiency of food which apparently weakens the resistance of the lower segments of the spinal cord to various toxic agents; though with proper nutrition these would be harmless. The particular symptoms of each outbreak depend on what particular toxic agent is present; these have been extensively studied, while some work has also been done on what particular ingredient of the diet is deficient. Considering the latter first, the

importance of vitamin A is stressed by Young [258], who not only found night blindness common in a village suffering from lathyrism, but also noticed that the disease did not occur in neighbouring villages where the diet contained as much *Lathyrus* but more vitamin A, fish and meat; while Shah [259] has reported great improvement in patients when vitamins A and D were given. Apart from night blindness no deficiency diseases have been reported as occurring with outbreaks of lathyrism, so that it seems improbable that lack of any vitamin, apart from vitamin A, is a factor. The nervous degenerations caused by vitamin E (p. 781) and those due to lack of some unknown factor reported by Wintrobe and others [175] do not, it is true, give any except neurological symptoms, but they are in essence progressive while lathyrism is a disease which never progresses beyond the initial paralysis. But, of course, lack of other substances in the diet apart from vitamins may be important, which is suggested by Basu and others [260], who found that the seeds of *Lathyrus sativus*, which often form the staple food in famine villages, are a very poor source of protein, being especially deficient in tryptophane. Minchin [261] believes that some protein deficiency may be the important factor, while McCarrison's experimental work with pigeons [262] on the effect of manurial conditions on the nutritive value of millet and wheat suggests that in areas where husbandry is poor grain may not only be less nutritious, but even toxic.

Of the factors which actually injure the already debilitated nervous system, and so are the immediate precipitating cause of lathyrism, some toxic substance in vetches of the *Lathyrus* family has for long been postulated. As early as 1770 an epidemic in France causing paralysis of the legs was thought to be caused by eating vetches, and a similar outbreak was seen in England in 1785, while in 1840 *Lathyrus cicera* was reported to cause paralysis when fed to rabbits [263]. In 1882 *Lathyrus cicera* or *clymenum* was held responsible for an outbreak in Syria which affected 1,200 people [264], and in the long French review of recent lathyrism in Syria *Lathyrus sativus* is held by Trabaud and his colleagues [264] to be the cause of lathyrism even when only a few seeds have been eaten. But they found this vetch harmless for the lower apes, camels, cows, fowls, rabbits and dogs; as did Basu [260] and others [265] for rats, and Anderson [266] for monkeys and other animals. None of these experiments, however, are really conclusive, since the animals were not on a famine diet analogous to the diets taken by men prior to developing lathyrism, nor was the vetch grown in the same soil as that around villages where lathyrism occurs. On the other hand, Anderson and others [266] showed that the seeds of a weed, *Vicia sativa*, and also an alkaloid extracted from them, affected the nervous system of monkeys and other animals, while Shah [259] investigating an outbreak of lathyrism found that seeds of *Vicia sativa* but not of *Lathyrus* had been eaten mixed with the corn. Young [258], however, reports that his cases had eaten *Lathyrus* but little or no *Vicia sativa*, and Minchin [261] describes "lathyrism without *Lathyrus*."

To sum up: it seems that lathyrism is due to lack of vitamin A and a

poor diet paving the way for toxic agents to attack the nervous system, these toxic agents not always being the same and therefore not always causing identical damage and symptoms. Probably they are sometimes alkaloids found in the seeds of *Vicia sativa*, sometimes alkaloids present in the seeds of various kinds of *Lathyrus* grown in particular soils, sometimes no clue is given as to their nature, so that one is forced to consider whether lathyrism may be due to an infection by some organism which is only pathogenic when the diet is impoverished.

Men are generally reported to be affected far more frequently than women [258, 261, 265], though not always [264], and the condition occurs at any age [258, 264], though mothers do not transmit the disease by suckling their children [264].

The clinical picture of lathyrism varies in every outbreak. Minchin [261], whose patients had eaten no *Lathyrus*, found that the onset of the paralysis of the legs may be sudden or slow; McCarrison [265] says the incubation period on bread containing *Lathyrus* is two to six months, while Traubaud [264] states that four days to four weeks after eating *Lathyrus* a tingling starts in the legs, which progresses to a tremor that is present at rest but changes to a spastic stiffness on walking. The patients may never be ill in themselves [261, 264], but Young [258] noted that there is a previous fever and Shah [259] that the condition begins with gastrointestinal symptoms.

Shah [259] reports that his cases had both spastic paralysis of the legs and sensory impairment and that occasionally the arms were involved, which also occurred in a few cases of Minchin's [261], but all other reports emphasize that there is a pure upper motor neurone involvement of the legs alone, so that the patients walk as if balancing along a rail [258] without any loss of their sense of position. The condition of the reflexes is very puzzling, since Minchin [261] observed that while the legs were spastic with extensor plantar responses, the cremasteric and abdominal reflexes remained normal, even in some cases where the arms were affected. Traubaud [264] found completely normal reflexes, including the plantar responses, though there was spasticity and clonus of the legs. The cerebrospinal fluid in new cases gives a paretic curve [261] and in old cases is normal [264].

Traubaud suggests that the damage is localized in the pyramidal tracts to the lower limbs because of changes in chronaxie. Minchin [261] noted that the bladder was occasionally affected, but other observers have not reported this.

Sexual impairment has been noted by earlier writers, but it is not mentioned in recent English reports from India, while the French observers [264] point out that lathyrism is an ideal disease for the Eastern male, since while it prevents him from working it in no way hinders him from enjoying those pleasures which are necessary for continuing his family tree. Neither pregnancy nor lactation are affected [264].

The disease is never progressive after a few days or weeks, and is generally considered incurable, though Shah [259] reports great improvement with cod-liver oil.

Other Effects of Lack of vitamin A. Frequent respiratory infections [111, 280] and diarrhoea [111, 230, 233, 249, 418] are the only symptoms, apart from those of the eyes and skin, which are frequently reported as occurring with a deficiency of vitamin A. Neither of these are of much value in diagnosis, though the cough is typical, being unproductive because of the blocking of the mucous glands by desquamated epithelia, and its cause can sometimes be confirmed by examination of nasal scrapings for metaplastic changes in the cells [111]. Children with xerophthalmia usually die of bronchopneumonia [230]. The diarrhoea has been cured by Pillat [249] and Sweet and K'Ang [111] with fats and vitamin A, which is reminiscent of Herfort's work (p. 25), who cured dyspepsia and diarrhoea with vitamin A.

Children with mild chronic deficiencies are lively and active [253, 254]

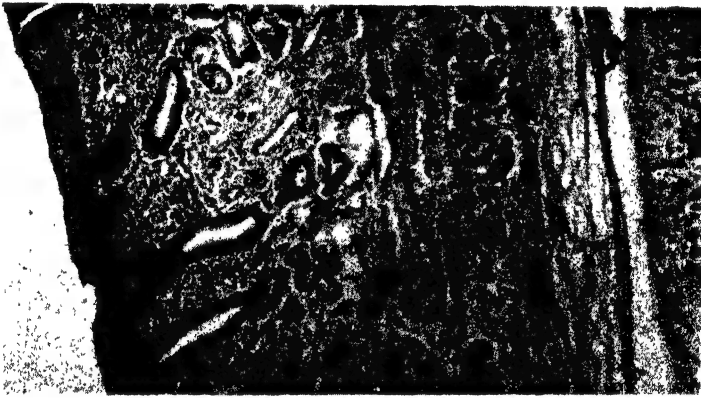


FIG. 23. Trachea of an American infant showing stratified keratinizing epithelium.

and have been described as podgy [249], but they are small for their age and have a high death-rate [254].

Post-mortem examinations in man (Figs. 3-7, 10, 23) emphasize that there is a great individual variation in which epithelial surface is most affected by lack of vitamin A, as indeed has been seen clinically by the frequency of either the skin (p. 68) or the eyes (p. 70) being damaged separately. This means that the diagnosis of a deficiency need not be discarded because all the typical changes are not present. Sweet and K'Ang [111] have carried out the largest number of post-mortem examinations of patients dying with a definite deficiency of vitamin A. Of seventeen cases eight had metaplasia of the epithelium of the larynx or trachea, which sometimes even involved the small bronchi and the mucous glands, whose ducts were blocked by the desquamated epithelium. In five cases the epithelium of the oesophagus was affected, though the rest of the digestive tract was normal, apart from the pancreatic ducts in one case. Changes were observed in the renal pelvises of three cases, but the ureters and bladders were normal, and so were the prostates, except one, of ten males. Of

seven females there were changes in the uterine mucosa of one. Hæmosiderosis was present in the liver and spleen of half the cases, but this was not regarded as being due to lack of vitamin A. In Wilson and Du Bois' child [185] the respiratory tract was extensively involved and so was the pancreas, the ducts being blocked by the shed cells and the acini cystic. The islets of Langerhans were normal, but in the thymus Hassall's corpuscles were enlarged. There was metaplasia of the renal pelvis, which was also noted in Boyle's infant [141] together with tracheal changes and defective tooth formation. The changes in the nervous system are described on p. 78; in the skin on p. 63; in the eyes on p. 70; and in the blood picture on p. 29.

The fundamental importance of vitamin A for epithelial surfaces is described on p. 28; its relationship to infections by bacteria on p. 28, and by intestinal worms on p. 29; its rôle in diabetes, thyroid and other endocrine diseases on p. 39; in pregnancy and lactation on pp. 15 and 50; in intestinal diseases including dyspepsia on p. 25; in hepatic disease on p. 54; in renal disease, renal secretion and hypertension on p. 37; in fever on p. 52; in tuberculous, leprosy, and simple ulcers and senile vaginitis on p. 32; and in the formation of the teeth on p. 26.

CAROTINÆMIA, XANTHOSIS CUTIS AND HYPERVITAMINOSIS A

Carotinæmia. This is a word which is loosely used to mean excess of carotene in the blood, though what constitutes an excess is unknown. Normal values (p. 57) are stated to be roughly 50 to 240 micrograms per 100 c.c., depending on the diet. When the carotene rises beyond a certain level in the blood, which varies with the individual, xanthosis cutis, or yellowness of the skin, occurs and as this is the symptom which draws attention to the excess of carotene in the blood, carotinæmia is increasingly used as a synonym for xanthosis cutis. This is incorrect as many patients with lipoid nephrosis or nephritis have a carotinæmia which does not discolour their skin though it would do so in normal people (p. 41). The explanation of this is obscure: it may be due to the glands of the skin failing to secrete carotene owing to their dysfunction caused by the nephritis or to the carotene being anchored in the abnormal nephritic blood (p. 20).

Carotinæmia without xanthosis cutis is probably a harmless condition, though Clausen [200] in a very thorough investigation showed that both very high and very low levels of carotene in the blood appeared to decrease slightly the resistance to respiratory infections. In animals Davies and Moore [88] could not produce a toxic state by giving huge amounts of carotene, although Sherwood and others [196] report that doses of carotene equal to about 1,500 I.U. of vitamin A stop œstrus and libido in rats.

Xanthosis Cutis. Stannus (885) in 1929 gave an extremely valuable review of clinical reports about this condition up to that date, while Joseph's paper [886] in 1944 should be read for its bibliography and summary of recent clinical and experimental work.

The causes of the high level of carotene in the blood which leads to

xanthosis cutis are pathological or dietetic; the former, which are mostly discussed elsewhere, include metabolic diseases, such as diabetes or myxœdema (p. 39), tuberculosis [385], disturbances in lipid metabolism (p. 41) and, possibly, failure to oxidize carotene [385, 386]. The dietetic cause of xanthosis cutis is simply the unduly high consumption of vegetables containing carotene such as happened in England in 1942 when the Ministry of Food, owing to a glut of carrots, advertised their virtues in every newspaper and even commissioned Walt Disney to draw the carrot family. In 1948 the carrot fly ruined the crop and propaganda and xanthosis cutis both declined.

The amount of carotene in the diet which causes xanthosis cutis varies with the individual. Two to three pounds of raw carrots weekly had been eaten by Thomson's patient [389] for eleven months, while an extra four pounds weekly for seven months was the smallest quantity which had been consumed by Almond and Logan's five patients [388]; this amount or its carotene equivalent in spinach had no effect on the colour of the skin after roughly two and a half months in Hoch's three experimental subjects [387], though double this amount did so within nineteen and twenty days in two subjects. Hoch noticed that the steady uniform rise in the blood carotene was checked when xanthosis cutis developed, presumably due to its storage in the skin. The level of vitamin A was raised to the upper limits of normal—126 to 155 I.U. per 100 c.c.—but not beyond.

The level of carotene in the blood at which xanthosis cutis develops differs widely in different people. Hoch [387] found in one experimental subject it was 375 micrograms and in another 470 micrograms per 100 c.c. In cases of established xanthosis cutis the levels have been between 220 and 610 micrograms per 100 c.c. [386], the former figure being within the so-called "normal" level (p. 57).

The curious canary yellow colour of the skin, which in severe cases becomes a deep orange, appears first in the palms of the hands and nasolabial folds [385, 386, 388] and in other areas where sweating is most marked or the horny layer of the skin is thickest, such as the forehead, axillæ and groins or the soles of the feet and knuckles. The nails may become dark and flecked with brown, and darkening of the eyebrows, but not of the hair of the scalp has been reported [385]. The mucous membranes and gums are not affected. This distribution of the colour is due to the staining of the horny layers of the skin by the carotene excreted by the sebaceous glands [386, 388]. The conjunctivæ are never discoloured and the urine and fæces are normal, which should prevent the usual mistake of diagnosing xanthosis cutis as jaundice [388, 389]. Sucklings develop xanthosis cutis when their mothers suffer from this condition [388, 389], and also infants and children [385] when fed on carrots from necessity during war [385, 390], or because of the foolish belief that carrots are good for children (p. 48). The colour of the skin may fade away within a little over two weeks of resuming a normal diet or may persist for many months.

Xanthosis cutis, when not a symptom of disease, is generally a harmless condition both in infants [386, 388, 389] and adults [386, 388],

though Henschen [387] reports a woman who, after eating two pounds of carrots daily, often scavenged from garbage, for six months developed a secondary anæmia and enlargement of the liver and spleen, which took many months to disappear after her diet became normal. Josephs [386] believes that high blood lipoids and cholesterol and a lowered basal metabolic rate are usual in chronic xanthosis cutis while weakness, loss of weight, leucopenia and a low blood pressure may also occur.

Hypervitaminosis A in Man. Little is known about the toxic effects of vitamin A in man. Rodahl and Moore [891], from chemical and biological investigations of the livers of polar-bears and seals, came to the conclusion that the severe illness which may promptly follow from eating these livers is due to intoxication from the huge amounts of vitamin A which they contain, since a helping of about three-quarters of a pound would provide 7,500,000 I.U. which, on analogy with rats, would be a toxic quantity. The symptoms of this acute polar-bear liver poisoning, which may start within two to four hours, are drowsiness, sluggishness, irritability or an irresistible desire to sleep, severe headache and vomiting. In severe cases peeling of the skin round the mouth starts after twenty-four hours and may remain confined to the face or the whole skin from head to foot may be involved. Rodahl and Moore also mention a man who took about 6,000,000 I.U. of vitamin A daily for five days—that is four to five ounces of halibut liver oil. He became severely ill, the main symptom being giddiness. The oil was stopped and he recovered within ten days. Getz [125] reports that 2,000,000 I.U. in a single dose may give a dull headache, and Spiesman [316] has seen adults who suffered from general malaise with loss of weight and appetite on only 40,000 I.U. daily. On the other hand, we have often given 144,000 I.U. daily for many weeks with no ill effects apart from an occasional and transient diuresis at the beginning of the treatment, and doses of 800,000 I.U. daily for several months appear to be harmless for children [199].

The very prolonged administration of vitamin A in large amounts may, however, be dangerous. Josephs [386] has very fully reported the case of a three-year-old boy who from the age of two or three months was given one teaspoon of halibut liver oil daily, that is about 240,000 U.S.P. units of vitamin A. His height was normal for his age, but his scalp hair was sparse, dry and coarse, and he had no eyebrows or hair on his body. He had slight clubbing of his fingers and toes. His liver was palpable 2 cm. below his costal margin, with a sharp firm edge; the spleen also was enlarged and firm, and palpable 8 cm. below his costal margin. X-rays showed that there was precocious development of the carpal centres of ossification, mottling of some epiphyses and thinning of the cortex of the small bones of the hands and feet. Laboratory investigations showed 910 U.S.P. units of serum vitamin A per 100 c.c., and greatly raised blood phosphatase and lipoids. After two and a half years without any additional vitamin A the boy was healthy and normal save for the persistence of the enlargement of the spleen and liver, the precocious skeletal development and the clubbing of the fingers and toes.

Even allowing for personal idiosyncrasy it seems unlikely that there is

any risk of poisoning with vitamin preparations, though it is as well to remember that some contain 72,000 I.U. per c.c. One case of allergy to vitamin A and carotene present in a normal diet has been reported [892].

Hypervitaminosis A in Animals. Moore and his collaborators (88, 187, 391) report that rats, when given about 27,000 I.U. daily, become limp and very emaciated, their hair rough and smeared with urine, their eyes swollen and encrusted and there is loss of hair round their mouths, which are sore and inflamed: the latter symptom may be due to the irritating effect of the oil, though it also occurs in man (p. 80). Pneumonia and enteritis may occur. The more specific lesions are softening and fractures of the bones with callus formation, seen most frequently in growing rats, and sudden and profuse internal hæmorrhages, often seen in adult animals. Since death may occur long before the liver is saturated with vitamin A, Moore and his collaborators conclude that it is due to too rapid absorption of vitamin A and not to a direct toxic effect of large stores of vitamin A on the liver. Strauss [198] found that the decalcification of the bones could not be prevented by vitamin D. Drigalski and Laubmann [197] report that in hypervitaminosis A there is also degeneration of the renal tubules and glomeruli, of the testes, of cardiac muscle and of hepatic cells to a smaller extent. Josephs [886] gives an extensive review of the literature which includes references to paralysis, to deposition of fat in the reticuloendothelial cells, to alterations in lipid and cholesterol metabolism (p. 41) and to changes in the endocrine glands.

VITAMIN A₂

Vitamin A₂ is chiefly found in fresh-water fish and is probably of little more than theoretical importance, since it appears to be made from the same vegetable precursors as vitamin A₁ and to have the same biological functions. Biological tests show it has a vitamin A activity of 47,500 U.S.P. units per gram [438].

Its structure, according to Gillam and others [269], is the same as that of vitamin A₁, except that in the place of the terminal $\text{—CH}_2\text{OH}$ group is a $\text{—CH=CH—CH}_2\text{OH}$ group, but Gray and Cawley [270] believe that the two vitamins have the same number of carbon atoms, the only difference being that vitamin A₂ has one additional unsaturated linkage. Hawkins and Hunter [348] do not consider that any of the formulæ so far suggested are correct.

The absorption spectrum of vitamin A₂ gives two bands with maxima at 350 and 288 millimicrons, and with antimony trichloride a band at 698, with subsidiary bands at 660 and 635–640 millimicrons [271], the latter, according to Lederer and Rothman [49], causing difficulty in the estimation of vitamin A₁ by overlapping its band at 620 millimicrons. Cyclized vitamin A₂ gives bands at 391, 369, 349, 334 millimicrons which are almost identical with those of cyclized vitamin A₁ [271, 272], but the latter is not so well adsorbed by alumina [272]. Cyclization does not alter the antimony trichloride spectrum of vitamin A₂ [272].

Vitamin A₂ is found in all fish liver oils, but those of fresh-water fish

contain most [271]. In the latter there appears to be a fixed ratio between vitamin A₁ and vitamin A₂, which is dependent on whether the fish is carnivorous, omnivorous, or migratory, but is not affected by age, sex, weight, or season [273]. Mammals, including man, birds and reptiles [274] do not store vitamin A₂ in their livers unless their diets, like that of the seal and otter which live on fresh-water fish, are very rich in the vitamin [269]. Rats and frogs have been found to store vitamin A₂ when it has been given in large quantities [273], and Milas [187] has reported finding it in the olfactory mucosa of the steer. The precursors of vitamin A₂ are probably the same as those of vitamin A₁, since Morton and Creed [275] showed that perch after being fed on leaf carotene formed both vitamins.

The functions of vitamin A₂ are apparently the same as those of vitamin A₁, since vitamin A₂ is biologically active when fed to rats [269], though it is more toxic [488]. Wald [276] found that it entirely replaced vitamin A₁ in the visual purple cycle of fresh-water fish. In migratory fish both vitamins are present in the eye, the proportion of vitamin A₁ being greatest in those fish which spawn in salt water [277]. Fluorescent microscopy is said to be able to differentiate between the two vitamins in the tissues [290].

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CHAPTER II

THE VITAMIN B COMPLEX

UNTIL 1926 it was generally believed that "vitamin B" was a single entity. In that year Smith and Hendrick [1] showed that it consisted of two factors, a thermolabile anti-neuritic factor and a thermostable growth promoting factor. After the dual nature of vitamin B had been demonstrated the American Society of Biological Chemists decided to call the factors *vitamin B* and *vitamin F* respectively. In England the names vitamins B_1 and B_2 were suggested. Vitamin B_1 was first isolated in 1926; its identity and synthesis took another ten years (p. 150). It soon became evident that vitamin B_2 , the thermostable factor, was a complex. The unravelling of this complex is shown in the synoptic historical table opposite.

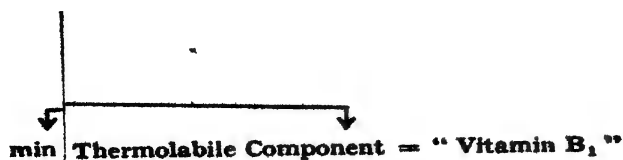
Considerable confusion has resulted over nomenclature. Thus the name vitamin B_2 was subsequently given to the vitamin now known as riboflavin, which is also called lactoflavine on the Continent, and was formerly known as vitamin G in America. The situation has been further complicated by the fact that workers in different laboratories have discovered factors independently and each group has given the factor its own name. For example vitamin B_6 has been successively known as factor Y, factor I, factor H, adermin and pyridoxine.

When the vitamins were isolated as chemical compounds many were given chemical names, indicating their nature, *e.g.*, pantothenic acid, riboflavin, pyridoxine, etc., and these are used in the literature instead of the older names. The term "vitamin B complex" now refers to all the vitamins split off from the original "vitamin B" and identified chemically or by their biological effects. The term "vitamin B_2 complex" is also used. This includes all the B vitamins except vitamin B_1 .

The components of the vitamin B complex are as follows :

Vitamin B_1 .	*Vitamins L_1 and L_2 .
Vitamin B_2 , now called Riboflavin.	*Vitamin M (Folic acid ? Vitamin B_c ?).
*Vitamin B_3 (Pantothenic acid ?).	*Factor U (vitamin B_c ?).
*Vitamin B_4 (a mixture of known amino- acids ?).	Nicotinic acid.
*Vitamin B_5 (Vitamin B_6 ?).	*Rice polish factor (a mixture ?).
Vitamin B_6 or Pyridoxine.	Folic acid (Vitamin B_c ?).
*Vitamin B_7 .	Inositol.
Vitamin B_8 or Adenylic acid.	Choline.
*Vitamin B_{10} .	Pantothenic acid.
*Vitamin B_{11} .	Biotin.
Vitamin B_c (Folic acid ?).	<i>p</i> -Aminobenzoic acid.

Those marked with an asterisk have not been identified chemically. Their existence is recognized from animal feeding experiments by which a nutritional deficiency syndrome is produced in the absence of the



min Thermolabile Component = "Vitamin B₁"

ascorbic
P.P. f

Filtrate
Factor

Pantothenic acid
isolation and synthesis

= anti-grey
hair factor

Vitamin B₁
isolated

Vitamin B₁
synthesised

1926

1927

1928

1929

1930

1931

1932

1933

1934

1935

1936

1937

1938

1939

1940

1941

1942

1943

1944

ION

supposed factor. Some may well be other known members of the vitamin B complex. This is probably true of vitamins B₃, B₄ and B₅, vitamin M, vitamins L₁ and L₂ and the rice polish factor.

Vitamin B₁. See p. 149.

Vitamin B₂. See Riboflavin, p. 306.

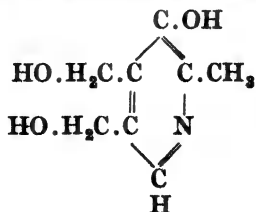
Vitamin B₃. In 1928 Williams and Waterman [8] observed that when adult pigeons were made polyneuritic by an exclusive diet of polished rice, addition of the antineuritic vitamin to the diet failed to restore the declining weight of the birds. The same diet with brewer's yeast brought about an increase in weight. They therefore postulated the existence of a new vitamin, later named vitamin B₃ by Williams and Eddy [4], essential for the growth of pigeons. It has never been isolated. It may be identical with pantothenic acid [9].

Vitamin B₄. This vitamin, the existence of which is very doubtful, is said to be concerned with the prevention of a specific type of paralysis in rats and chicks [6, 7]. Like vitamins B₃ and B₅ it may well consist of other known factors. Recent work identifies it with the amino-acids arginine, glycine and cystine [185].

Vitamin B₅. In 1930 Carter, Kinnersley and Peters [8] described an alkali-heat-stable, water-soluble factor present in yeast, essential for the weight maintenance of pigeons, but it is now thought to be identical with vitamin B₆ [9].

VITAMIN B₆ OR PYRIDOXINE (RAT ANTIDERMATITIS FACTOR, ADERMIN)

Isolation and Chemistry of Vitamin B₆. In 1934 György reported the existence of a factor distinct from the water-soluble factors known at that time, lack of which caused dermatitis or acrodynia in rats. It was called vitamin B₆ and was later shown to be identical with the factor Y of Chick and Copping [68], the antidermatitis factor of Hogan and Richardson [64], the "vitamin H" of Booher [65] and the factor I of Lepkovsky, Jukes and Krause [66]. The vitamin was isolated in 1939 by a number of investigators [69-71], and its structure determined in the same year [72-75]. It is 2-methyl-8-hydroxy-4 : 5-dihydroxymethyl pyridine,



and like nicotinic acid is a pyridine derivative. The synthesis of vitamin B₆ was effected in 1939 by Harris and Folkers [76] in America and by Kühn [77] and his co-workers in Heidelberg.

The name of the vitamin was subsequently altered to adermin by Kühn in 1938, and in the following year György and Eckhardt [79] proposed that it should be called pyridoxine, a name which was adopted in 1940 by the Council on Pharmacy and Chemistry of the American Medical Association [80].

Vitamin B₆ forms colourless crystals, M.P. 160° C., soluble in water and alcohol, stable to heat and alkali, but not to light, especially ultra-violet. It is more susceptible to the action of light in neutral and alkaline media [186]. The *pH* of a one per cent. solution of the hydrochloride is 2.44.

Various methods have been devised for the estimation of vitamin B₆.



FIG. 24. Crystals of Vitamin B₆ (Pyridoxine).

One, due to Swaminathan [139], is based on the colour reaction between vitamin B₆ and diazotised sulphanilic acid. A modification of this has been suggested by Bina and his co-workers [187]. Biological methods depending on the cure of rat acrodynia [81], the growth rate increase of rats [95, 188], and the growth response of yeast [189, 190], or moulds [191] have been devised for its estimation.

Distribution of Vitamin B₆ in Foods. Vitamin B₆ appears to be widely distributed in foods. Yeast, liver, cereal polishings, cereals and pulses are particularly good sources. There is an increase of this vitamin in cereals on germination. Fish is a moderately good source; vegetables and milk contain little vitamin B₆. Vitamin B₆ bound to protein is not completely absorbed, therefore food should be cooked if all the vitamin is to be assimilated [192].

Vitamin B₆ Content of Foodstuffs [5]

	Micrograms per gm.
Apples	0.26
Bananas	3.2
Bean, dried	5.5
Beef, muscle	0.77
liver	1.7
heart	1.2
Beets	1.1
Cabbage	1.2
Carrots	1.2
Cauliflower	0.20
Cheese	0.66
Chicken, leg	0.25
breast	1.3
Chocolate	0.23
Corn meal, white (maize)	0.54
Eggs	0.22
Grape-fruit	0.09
Halibut	1.1
Lamb, leg	0.81
Milk, new	0.06
dried	0.50
Molasses	2.7
Mushrooms	0.45
Mutton, shoulder	0.18
Onions	0.63
Orange	0.8
Oyster	0.33
Peas, fresh	0.79-1.9
dried	3
Peanuts, roasted	3
Pork, loin	0.86-2.7
bacon	0.29-1.0
ham	0.19-1.7
Potatoes	2.2-3.2
Raisins	0.94
"Royal jelly"	2.4
Salmon	0.33
Spinach	0.83
Strawberries	0.44
Tomatoes	0.6
Turnips	1.1
Veal	0.56-1.8
Wheat, whole	4.2*
National wheat flour	3.1*
white flour	1.8*
wheat germ	6-17.5
Yeast (dried)	36

* Ministry of Food figures [371].

Physiology of Vitamin B₆. Rats fed on diets deficient in vitamin B₆ develop cutaneous symptoms characterized by a symmetrical dermatosis

affecting first the paws, then the tips of the ears and nose, which become red, swollen and oedematous. The matted fur on the backs of the hind paws desquamates, leaving a denuded, pale pink, glistening skin. Sometimes there is fissuring or ulceration at the angles of the mouth, with a lesion at the tip of the tongue, which may ulcerate. Dann [122], however, states that in his experiments the majority of rats on a diet deficient in vitamin B₆ developed dermatitis, but a large number failed to do so. Others claim that rat dermatitis can be cured by rice bran concentrate, containing vitamin B₆ and a second accessory factor, vitamin B₆ alone being ineffective [128]. This secondary factor has been identified as the rice polish factor [84].

György [85] noted that the dermatitis of vitamin B₆-deficient rats was much worse in cold weather. He states that there is a striking similarity between rat acrodynia and chilblains, and suggests that possibly a vitamin B₆ deficiency in man may predispose to chilblains. Not only is vitamin B₆ associated with dermatitis in the rat, but when the animal is fed on a diet containing it there is greater growth and deposition of body fat than in controls not receiving the vitamin in spite of the fact that the food intake is identical in each case. Vitamin B₆ therefore appears to effect a more efficient utilization of the food [86]. Animals on diets deficient in the vitamin show a fall in metabolic rate [198].

The relationship of vitamin B₆ to some of the unsaturated fatty acids is of interest. The unsaturated fatty acids of maize oil are effective in alleviating the symptoms of vitamin B₆ deficiency in rats [87], and it is therefore thought that both vitamin B₆ and fats are needed to prevent rat dermatitis [107]. Schneider [128] and his collaborators claim that it can be cured by "essential fatty acids," such as linoleic acid, independently of vitamin B₆, which alone has only a temporary effect. The connection between vitamin B₆ and fat metabolism has been studied by Gavin and McHenry [96], who noted that the administration of vitamin B₆ in conjunction with vitamin B₁, riboflavin and choline to rats fed a fat-free diet caused a slight increase in body fat and an increase in body weight. The effect was augmented by nicotinic acid. The same workers also suggest that vitamin B₆ is essential for the metabolism of protein [168].

Chick [89] and her co-workers have observed that if rats are maintained for periods of more than four months on a vitamin B₆ deficient diet they suffer from fits of an epileptiform nature. These fits, which can be prevented and cured by the daily administration of the vitamin in doses of 10–15 μ g, showed a close resemblance to those previously observed in young pigs kept on a diet deficient in vitamin B₆ [90]. This has been confirmed [840]. It would thus appear that the normal activity of the motor cells of the cerebral cortex, in certain species at any rate, depends upon an adequate supply of the vitamin. The pigs also developed a microcytic hypochromic anaemia, curable by administering vitamin B₆. Wintrobe [194] has also described fatty changes in the livers of such pigs, as well as ataxia and degenerative changes in the spinal cord and peripheral nerves [195]. There also appears to be a disturbance in tryptophane metabolism in the pig [864]. Anaemia has been induced by Fouts [92, 98] and his co-workers and by McKibbin and his colleagues [164] in puppies and dogs by

feeding them on diets containing all the essential vitamins but lacking vitamin B₆. This anæmia is cured by crystalline vitamin B₆ in doses of 60 μ g per kilogram of body weight. Borson and Mettier [112], however, claim that whereas vitamin B₆ relieves hypochromic anæmia in dogs, an adequate supply of the non-adsorbable fraction of the vitamin B complex is required as well for complete cure of the anæmia. These observations establish a possible link between the formation of hæmoglobin and the vitamin, although clinical studies by Kark, Lozner and Meiklejohn [94] showed that the vitamin was ineffective in curing idiopathic hypochromic anæmia and nutritional macrocytic anæmia in patients suffering from alcoholic or endemic pellagra with associated anæmia. They were given ferrous sulphate, nicotinic acid, riboflavin, vitamin B₁ and vitamin B₆ in amounts varying from 880 to 1,140 mg.

It has been shown that vitamin B₆ is not only required by the rat, but is also essential for the nutrition of the dog, chick, pigeon, and for the growth of yeast and a number of bacteria [98-106]. The intestinal micro-organisms of some animals appear to possess the power of synthesizing the vitamin [180].

Cowgill [140] has examined the effect of vitamin B₆ deficiency in the dog. A severe anæmia, curable by administering vitamin B₆ but not iron, develops after the animals have been between a hundred and twenty and three hundred and twenty days on a diet free from the vitamin. After three hundred days or more some of the dogs develop marked cardiac embarrassment, including dyspnœa, tachycardia, dilatation and hypertrophy of the right ventricle and auricle, and passive congestion of the liver. Degenerative changes in the myelin sheaths of the peripheral nerves and the spinal cord also occur.

The degree of phagocytosis by the leucocytes in the blood of animals deficient in vitamin B₆ is reduced according to Cottingham and Mills [345]. They found that the leucocytes in the blood of rats receiving a diet adequate in vitamin B₆ took up an average of 13.56 test organisms (*Micrococcus candidus*) per cell in four minutes, and by the end of an hour eighty-two per cent. of the cells showed evidence of intracellular digestion. On a diet deficient in vitamin B₆ this was reduced to 4.95 organisms per leucocyte and intracellular digestion was noted in only fifteen per cent. of the latter.

Studies have been made on the toxicity of vitamin B₆ [111]. It has been shown that rats tolerate doses up to 1 gm. per kilo of body weight, but above this amount toxic convulsions appear. The lethal dose for the rat is 4 to 6 gm. by mouth per kilo. The repeated administration of 100 mg. per kilo of body weight is well tolerated by small animals for two weeks without any untoward effects. When given intramuscularly to human beings it causes a certain amount of pain on account of the acidity of the solution.

Vitamin B₆, like riboflavin and nicotinic acid, probably forms part of an oxidation-reduction enzyme system in the body. *In vitro* it can act as a hydrogen acceptor. The requirements are increased in hyperthyroidism [162].

The toxic symptoms produced by the sulphone drug, promin (anæmia, loss of weight, anorexia, irritability), do not appear if large doses of vitamins B₆ and B₁ and riboflavin are given [361].

Clinical Studies on Vitamin B₆. Excretion. Studies in the human excretion of vitamin B₆ have been made by Spies [119] and his co-workers, using a method of estimation devised by Scudi, Unna and Antopol [120]. Within an hour following the intravenous injection of 50 mg. of vitamin B₆ the average urinary excretion is about 8.7 per cent. When the same dose was given to nine patients with evidence of pellagra, beriberi or riboflavin deficiency, the excretion only averaged 0.5 per cent. with a range of 0.0 to 1.5 per cent. Four of the patients suspected of having a vitamin B₆ deficiency excreted an average of only 0.2 per cent. Three patients with deficiency diseases who were hospitalized and restricted to a diet deficient in vitamin B₆ excreted practically none of the injected vitamin B₆. The normal urinary excretion is between 400 and 750 µg in twenty-four hours [111, 166]. According to Scudi [165] the vitamin is excreted as a conjugate, probably a glucuronide or ethereal sulphate, although Huff and Perlzweig [341] state that it is excreted as the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxy methyl pyridine [341]. In doses of 100 mg. vitamin B₆ produces a bluish purple fluorescence in the urine [205]. The fluorescent product is 2-methyl-3 hydroxy-4-carboxy-5 hydroxy methylpyridine [365]. After a dose of 50 mg. of vitamin B₆ by mouth 3 to 8 mg. of this substance is excreted in four hours.

Flexner and Chassin [88] administered 50 mg. of vitamin B₆ in 400 c.c. of water to eighty-four patients and examined the urinary excretion of the vitamin. Patients under fifty excreted 8.4 per cent. Thirteen out of thirty-two over fifty had an output of only 2.3 per cent., while the remainder showed a normal excretion. Between the ages five and fifteen the excretion was 21.3 per cent. of the dose given. Patients with post-encephalitic parkinsonism showed a diminished output, averaging 2.5 per cent. of the dose.

It has not been shown that vitamin B₆ is essential for human nutrition. If it is, the probable requirements, calculated from animal data, are of the order of 1.5 to 2 mg. daily.

Pellagra and Beriberi. Spies, Bean and Asche [108] studied a number of undernourished patients who had clinical evidence of pellagra and beriberi. They were considerably benefited by the administration of nicotinic acid, vitamin B₁ and riboflavin as supplements to their diets. In cases where the diet was improved as well the patients made an excellent recovery. But those who still lived on inadequate diets developed symptoms such as extreme nervousness, insomnia, irritability, abdominal pain, weakness, and difficulty in walking. These symptoms were not relieved by the vitamins previously mentioned.

Four of these patients on deficient diets were selected and given injections of 50 mg. of pure synthetic vitamin B₆ in sterile normal saline. Within four hours all had experienced relief and increased strength and within twenty-four hours the symptoms had disappeared. One of the patients, who previously was unable to walk more than a few yards, walked two miles a day after receiving 50 mg. of vitamin B₆. This study

was followed up and twenty more patients were treated with similar results [109].

Anæmia. Following the observations of Fouts and his co-workers on the occurrence of hypochromic anæmia in vitamin B₆ deficient dogs Vilter, Schiro and Spies [110] gave large amounts of the vitamin to pellagrins with macrocytic anæmia and to patients with pernicious anæmia. Three pellagrins with macrocytic and two patients with pernicious anæmia were given 50 to 100 mg. of pure vitamin B₆ intravenously for a period of ten days. Within two days they experienced considerable increase in well-being and strength. On the fifth, sixth, seventh and eighth days a slight but definite increase in reticulocytes was seen in each case. The reticulocytes did not rise above five per cent., but the white cell count, which was low in the pernicious anæmia patients, rose considerably, particularly the polymorphonuclear leucocytes. 100 mg. of vitamin B₆ incubated with 100 c.c. of fasting normal gastric juice was given by mouth to one of the pernicious anæmia patients after reticulocytes and white cells had fallen to their original low level, with a response identical with that observed by injecting the vitamin B₆ intravenously. Spies states that vitamin B₆ has a definite sedative effect when administered intravenously in large amounts. An increase in the erythrocyte count and hyperplasia of the bone marrow has been recorded in three cases of primary erythroblastic anæmia (Cooley) treated with vitamin B₆ and pregnancy urine hormone [18]. Kark [94] and his colleagues failed to observe any improvement in six anæmic patients after giving vitamin B₆.

Neuromuscular and Nervous Diseases. The finding of foci of muscle atrophy in vitamin B₆ deficient rats, and the production of tremors and

vitamin [111], suggested that it might have some effect on muscle action. Accordingly Antopol and Schotland [114] gave it intramuscularly in doses of 100 to 500 mg. weekly in six cases of pseudohypertrophic muscular dystrophy and they reported a considerable improvement in the condition of all the patients; no untoward symptoms were observed in any of the cases. One patient had previously failed to respond to glycine or prostigmin therapy. Another showed improvement within half an hour of an injection of 50 mg. of vitamin B₁. Spies [109] states that improvement was noted after giving vitamin B₆ intravenously to patients with idiopathic epilepsy, amyotrophic lateral sclerosis and myasthenia gravis. Three cases of the latter showed distinct improvement within twenty-four to forty-eight hours after the injection of the vitamin. Careful investigation by a number of workers, including the authors, has shown that these claims for vitamin B₆ are premature and that it is ineffective in the treatment of muscular dystrophy and amyotrophic sclerosis [97, 168, 169, 174, 196, 197, 198, 199]. McCollum [129] has shown that vitamin B₆ is quite valueless in the prevention and treatment of nutritional muscular dystrophy in rabbits.

Spies [109] has used vitamin B₆ in eleven selected cases of Parkinsonism of at least four years' duration, eight of the cases being arteriosclerotic and three post-encephalitic. In the latter considerable improvement was reported within a few minutes; rigidity was significantly decreased and the patients walked without their usual stiffness. Two of

the arteriosclerotic patients showed definite improvement, five were unchanged and one was considerably worse. From a study of forty-six cases Jolliffe [115] concluded that no improvement occurred in postencephalitic cases and little or no improvement in patients hospitalized for over three years, but that a dramatic improvement resulted in approximately twenty per cent. of cases of non-postencephalitic Parkinsonism that had been helpless for less than a year. All patients received 50 to 100 mg. of vitamin B₆ intravenously. Jolliffe [200] subsequently extended the series to ninety cases and reported permanent improvement in nine, *i.e.*, ten per cent. Baker [142] has also observed improvement in eight cases out of nineteen and Meller [171] in nine cases out of ten receiving a similar dosage. They both state that in idiopathic paralysis agitans vitamin B₆ therapy decreases pain and rigidity. Three cases of paralysis agitans were also successfully treated by Rudesill and Weigand [172] with doses of 50 to 100 mg. of vitamin B₆ daily for five to seven months. They noted subjective and objective improvement in rigidity, tremor and strength. Zeligs [148], however, treated fifteen cases and Barker and his colleagues [173] ten cases of Parkinsonism with daily intravenous injections of 50 to 100 mg. of vitamin B₆ and observed no beneficial effects whatsoever. Loughlin [201] also failed to observe any benefit in twelve chronic cases. The reported material is still too meagre for final appraisal, but even accepting Jolliffe's latest report it would appear that vitamin B₆ therapy is only effective in ten per cent. of patients with paralysis agitans, a low figure that makes its use hardly worth while.

Vilter, Aring and Spies [116] have reported a case of arsenical peripheral neuritis that had been treated with vitamin B₆ after unsuccessful treatment with vitamin B₁. The patient had swallowed arsenic trioxide and after two weeks he showed the signs associated with arsenical neuritis—muscle cramps, weakness of muscles of the hand, hyperæsthesia of the hands, arms, foot and leg, anæsthesia to light touch, diminished superficial reflexes, and absent deep reflexes. He was treated for three weeks with daily intravenous injections of 50 mg. vitamin B₁ without any effect. This was discontinued and he was given 50 mg. of α -tocopherol intramuscularly twice daily for a week without any improvement. Then 20 mg. of vitamin B₆ was given intravenously twice a day for three days. Within four hours of the first injection subjective improvement was noted. During the three days there was a striking increase in muscular power, as measured in dynamometer units, and a decrease in hyperæsthesia, deep pain and cramp. A relapse occurred when the vitamin B₆ was substituted by normal saline or vitamin B₁. Vitamin B₆ was the only substance that caused a remission, and its action was more spectacular when α -tocopherol was given as well. After ten weeks the patient had completely recovered.

Stone [860] claims that several intraspinal injections of 30 to 50 mg. of vitamin B₆ were effective in relieving twenty-six patients with diseases of the nervous system. The conditions included Sydenham's chorea, infectious meningomyeloradiculitis, anterior poliomyelitis, Korsakoff's syndrome, disseminated sclerosis and spastic paraplegia. The number of cases of each condition studied was very small, and spontaneous improvement or a period of remission cannot be excluded as no controlled observa-

tions were made. Stone states that in a case of anterior poliomyelitis relaxation of muscular rigidity, improvement in muscle strength and increase in the range of active and passive movements occurred twenty-four hours after injecting 50 mg. of vitamin B₆ intraspinally.

Vitamin B₆ has been used by Schwartzman and his co-workers [170] in the treatment of Sydenham's chorea in a total dosage of 180 mg. to 840 mg.; the daily dosage was from 9 mg. to 60 mg. a day. Improvement was noted after several days, and was progressive. The response of the patients was said to be dramatic. As only three cases were treated, spontaneous recovery cannot be excluded.

Cheilosis. The use of vitamin B₆ in the treatment of cheilosis (p. 329), which is usually associated with riboflavin deficiency, has been reported [117, 202], daily doses of 20 to 100 mg. being given intravenously. In one case there was slight but definite objective improvement in five hours, and in twenty-four hours the improvement was demonstrable in a photograph. When the vitamin was withheld there was slight regression. In all cases the lesions cleared up within five days of treatment. Only three patients showed improvement when given riboflavin. Machella [167] states that cheilosis is not necessarily a manifestation of riboflavin deficiency; he has treated a number of cases with vitamin B₆ without riboflavin. It is possible that riboflavin deficiency is primary and specific in the ætiology of cheilosis and that vitamin B₆ is an adjuvant in treatment, or that the rôles of the two vitamins are reversed. On the other hand, they may both be essential for the nutrition of the lips at the mucocutaneous junctions, and deficiency of either of them precipitates the lip lesions.

Skin Diseases. Pehl [118] reports on the administration of vitamin B₆ in doses of 5 to 20 mg. to eight infants aged one to six months, suffering from nutritional upsets and seborrhœic skin troubles. After several days' treatment no improvement was noted in six cases, and the other two showed slight improvement, which might have been due to treatment. No toxic effects were observed.

Since a deficiency of vitamin B₆ is associated with dermatitis in the experimental animal, attempts have been made to use it clinically in dermatological conditions. Jolliffe [203] claims to have had some success with oral doses of 50 to 250 mg. daily in the treatment of thirty-seven patients with adolescent acne vulgaris. Nine were cured and nineteen improved; in a control series seven out of thirty-five improved. In many of the patients a marked reduction in the oiliness of the skin, even in some to the point of actual dryness of the skin, was stated to have occurred.

Wright and his colleagues [204] describe the treatment of an unspecified number of patients suffering from seborrhœic dermatitis and eczematous eruptions of unknown ætiology with 25 to 100 mg. of vitamin B₆ parenterally. The number of injections and the period over which they were given is not given, but on the basis of "clinical impression" the patients showed a rapid response.

Other Conditions. There are reports on the treatment of nausea and vomiting of pregnancy with vitamin B₆. These have not been adequately controlled. The importance of controlled studies in such patients, who are

very susceptible to suggestion, should not be forgotten. Nor should the beneficial effects of sedation and dietary supervision be overlooked in these cases. Weinstein [206] reports on a series of sixty-eight patients given 50 to 100 mg. of vitamin B₆ intramuscularly three times weekly or 10 to 20 mg. orally three or four times daily. The total dosage varied from 150 to 2,500 mg. Of thirty-seven patients with morning nausea thirty-four were stated to be relieved completely; six patients with nausea and vomiting that prevented retention of food were all relieved. Willis [207] treated thirty-seven patients with doses of 50 mg. of vitamin B₆ up to a total dosage of 1,500 mg. with apparently good results in most cases. Very satisfactory results are also reported by Hart and co-workers [342], who state that the simultaneous injection of vitamin B₁ gives better results than vitamin B₆ alone. Both vitamins were given in doses of 50 to 100 mg. intravenously. Clark [184] claims that vitamin B₆ is effective in the treatment of hypocyclusis. Twelve patients were given 50 mg. of vitamin B₆ intramuscularly for six days, then every third day for six doses and then weekly for six doses and then weekly for six weeks. Improvement is stated to have occurred in ten. There is no rationale for the use of vitamin B₆ in hypocyclusis.

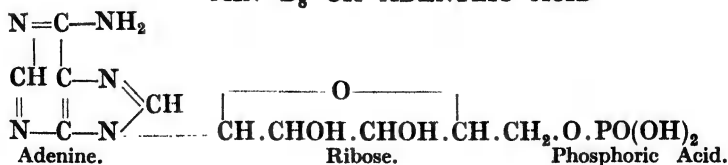
Substances rich in the vitamin B complex, such as liver extract, have been used for the treatment of irradiation sickness, and more recently isolated factors of the complex have been employed. Maxfield and co-workers [346] claim that a single intravenous injection of 25 mg. of vitamin B₆ relieves irradiation sickness in the majority of cases. If necessary the dose is repeated at twenty-four to seventy-two hour intervals. Gratifying results were stated to have been obtained in a series of over fifty cases.

Further controlled clinical investigations are needed before the therapeutic value of vitamin B₆ can be assessed, but the results obtained so far are disappointing.

Vitamin B₇

This is the name given to a substance present in rice polishings, in the absence of which pigeons develop digestive disturbances [208]. It has never been isolated.

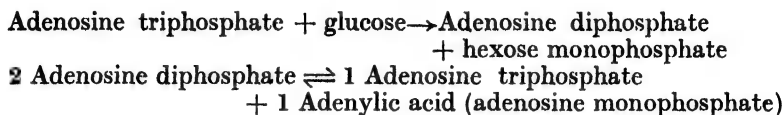
MIN B₈ OR ADENYLIC ACID



Adenylic acid or adenosine monophosphate, a complex of adenine, ribose, and phosphoric acid, is widely distributed in nature, in cereals, glandular tissues and yeast from which it can be extracted. It is a constituent of the di- and tri-phosphopyridine dinucleotides, codehydrogenases I and II (p. 381), which are "respiratory mediators" forming part of the

enzyme system concerned in catalysing the oxidation-reduction mechanisms that occur in the living cell. According to Ochoa [126] adenylic acid is essential for the phosphorylation of glucose, adenylic acid being first converted into adenosine triphosphate, which transfers its labile phosphate to glucose.

The mechanism of phosphorylation probably occurs according to the following scheme :—



According to Kalckar [209] the reaction is catalysed by myokinase, an enzyme present in skeletal muscle. There is evidence that the energy of muscular contraction is derived from the breakdown of adenosine triphosphate, which is catalysed by calcium ions. Certain adenine nucleotides also act as growth factors, causing increased proliferation following damage to cells [210]. Adenylic acid inhibits bacterial growth [211].

Following the claim of Reznikoff [220] that adenine sulphate is effective in the treatment of agranulocytic angina, Ruskin [212] used iron adenylate for the treatment of five cases of agranulocytosis following chemotherapy. All recovered. This resembles pentose nucleotide therapy. It is stated that adenylic acid enhances the effect of vitamin B₁ in cases refractory to treatment with the latter alone [218].

Spies [45, 46] and his collaborators administered adenylic acid to normal persons and to undernourished patients who had symptoms unrelieved by vitamin B₁, riboflavin, nicotinic acid, vitamin B₆ or vitamin A. The injection of from 8 to 20 mg. of adenylic acid in sterile normal saline produced within fifteen seconds involuntary, deep, gasping inspirations, a feeling of fulness in the head and a fluttering sensation in the upper part of the abdomen. The pupils became widely dilated and transient flushing of the neck and face occurred. The symptoms, however, disappeared within a few minutes and produced no permanent ill effects. Similar, although less intense symptoms followed the administration of a single oral dose of 200 mg. Six patients suffering from malnutrition and intense burning of the oral mucous membranes, but with no definite evidence of pellagra, received relief following the administration of adenylic acid, although they did not respond to nicotinic acid. When given orally in doses of 500 mg. for three days it also increased the effectiveness of nicotinic acid in the treatment of a series of pellagrins in relapse. In seven other cases of pellagra 1 gm. of adenylic acid daily produced gradual if not dramatic improvement. Spies, however, warns against the intravenous use of adenylic acid owing to the severe reactions produced. These studies suggest that adenylic acid may relieve some of the symptoms of mixed vitamin deficiencies.

These investigations were continued by Spies and his co-workers [158], who treated a further thirty patients, suffering from malnutrition, pellagra and peripheral neuritis, with adenylic acid. The ulcers in the mouths of

six malnourished individuals disappeared within two to five days following the intravenous administration of 50 mg. of adenylic acid twice daily. The ulcers of controls in whom there was no evidence of dietary deficiency failed to respond to similar treatment. Twenty patients with pellagrous glossitis and sub-clinical pellagra also showed rapid improvement in strength and the rapid disappearance of the burning sensations of the mucous membranes. The intravenous administration of adenylic acid to six persons with peripheral neuritis who failed to respond to yeast and large doses of vitamin B₁ was followed by striking clinical improvement after ten days to three weeks. Electrocardiograms showed that adenylic acid given intravenously produces a temporary tachycardia, prolonged PR and QT intervals, and inverted T waves. These changes disappeared five minutes after the injection.

If we accept the work of Spies and his collaborators, adenylic acid certainly has a powerful pharmacological action, but evidence for its status as a vitamin in human nutrition is lacking.

Vitamins B₁₀ and B₁₁

Vitamins B₁₀ and B₁₁ are two chemically unidentified water soluble members of the vitamin B complex stated to be necessary for growth and proper feather development in the chick [218]. It has been suggested that vitamins B₁₀ and B₁₁ are identical with vitamin B_c [357].

Vitamin B_c

This is also known as the chick anti-anæmic factor. Under certain dietary conditions chicks refuse to grow, and develop an anæmia, characterized by a decrease in percentage hæmoglobin and red cell volume, which is curable by liver extracts [214, 215], and with pure vitamin B_c [331].

This was recognized by Hogan and Parrott [214] as an unidentified member of the vitamin B complex and designated vitamin B_c. Piffner and his colleagues [216] have now isolated the vitamin in crystalline form from liver and yeast [357]. It forms orange-yellow platelets, melting at 860° C. Subsequent investigation has shown that it is a nitrogen-containing acid, destroyed by mineral acids at a pH 1. It can be adsorbed from acid solution and eluted by ammonia [217]. According to recent work vitamin B_c is identical with folic acid [336].

Vitamin B_c given orally protects rats against the hypochromic anæmia induced by the sulphone drugs promin and promizole [332].

Vitamin L₁ and L₂

In 1938 Nakahara [27] and his colleagues claimed that two factors were essential for the lactation of young rats. These were named L₁ and L₂, and were stated to be present in beef-liver extract and yeast respectively. It has been suggested that this vitamin complex may be identical with the liver filtrate factor of Morgan and Simms [28], necessary for the lactation of young rats, or with a factor postulated by Sure [29] present in the filtrate factor. Folley and others [145] could not confirm its existence.

Vitamin M

This vitamin, lack of which is said to produce cytopenia in monkeys [26, 128], may be identical with folic acid [219]. It is present in yeast and liver extract. Monkeys on diets deficient in the vitamin develop oral lesions resembling those of pellagra, and a lowered resistance of the intestinal mucosa to infection by *Bact. dysenteriae* (Flexner and Shiga). For this reason it has been argued that a nutritional deficiency may be an ætiological factor in human bacillary dysentery. There is possibly some relationship between vitamin M and the precursor of folic acid [370].

Factor U

This factor is a water-soluble growth factor required by chicks [41]. It occurs in yeast, wheat, bran and corn. It may be identical with vitamin B₆ [357].

Rice Polish Factor

This is a recently discovered factor present in rice polishings, essential for the growth and maintenance of experimental animals receiving all other known vitamins or factors. It is suggested that it may complement vitamin B₆ in preventing rat dermatitis [84]. The rice factor itself may be a complex, as it can be replaced by a mixture of glycine and glycuronic acid or certain pentoses [121].

Nicotinic Acid. See p. 375.

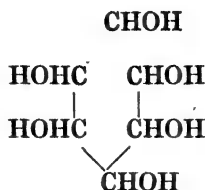
FOLIC ACID

Folic acid, so called because it occurs in green leaves, is also present in animal tissues, such as liver and kidney, and in mushrooms and yeast. It can be adsorbed from liver extract and eluted from the adsorbate, from which it has been isolated in crystalline form [335]. It is acidic in nature, contains nitrogen and has a molecular weight of about 500 [221]. Folic acid is a growth factor for rats [222], dogs [223], and certain bacteria [224]. Minute quantities have a powerful growth stimulating action [225] although it inhibits tumour growth [334]. Synthesis of folic acid occurs in the intestine of the rat [225], and sulphonamides such as succinylsulphathiazole (sulphasuxidine) interfere with its production in this organ [226]. The granulocytopenia and leukopenia produced by feeding laboratory animals with sulphaguanidine and succinylsulphathiazole (sulphasuxidine) can be prevented by administering crystalline folic acid [336, 372], the level of which in the liver falls considerably when these drugs are given [337]. It is possible that folic acid and biotin (p. 121) play some part in the utilization of pantothenic acid [227] and of vitamin K [226]. It has been suggested that folic acid is related to xanthopterin, the yellow pigment of butterfly wings [228, 229, 343]. Thus when synthetic xanthopterin is incubated with liver tissue a significant increase in folic acid occurs. More recent work identifies folic acid with vitamin B₉ [335, 336]. It is not known whether folic acid is essential for human nutrition. The average daily intake is 1.4 mg. per day [230]. The average daily urinary excretion is only 0.01 mg. [347]; this is so low in comparison with the

intake that it seems likely that it plays a rôle in human metabolism. Severe losses in folic acid occurs when foods are cooked, *e.g.*, from forty-six to ninety-five per cent. in meat products and sixty-nine to ninety-seven per cent. in vegetables [292].

INOSITOL

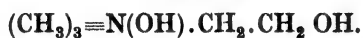
This is cyclohexanehexol :—



It is a normal constituent of all plant and animal tissues. Fruits, cereal grains, yeast and other moulds and bacteria are good sources of inositol. In plants it is mainly present as the hexaphosphate (phytic acid). It also occurs in muscle, liver, kidney, blood, milk and eggs. In the cooking of food twenty-five to fifty per cent. of the inositol is inactivated. Little is known of the rôle of inositol in animal physiology. It has been suggested that with pantothenic acid it is essential for normal gastro-intestinal function. Martin and his co-workers [151] have shown that dogs kept on a diet deficient in inositol and pantothenic acid show increased gastric emptying time with pylorospasm, marked segmentation of both small and large intestine, gastro-intestinal hypertonicity and hypomotility, alternation of ribbon-like segments in the bowel alternating with dilated loops and frequent gas formation. The conclusions were based on X-ray studies. Martin suggests that some of the gastro-intestinal symptoms of deficiency diseases such as pellagra and sprue, and those produced by experimentally produced vitamin B complex deficiency in man (p. 281), may be due to deficiency of inositol and pantothenic acid. He also claims that inositol is a nutritional factor controlling gastro-intestinal motility in mice. Others have reported that inositol increases the intestinal motility of dogs [281], and that patients with atrophic gastritis show some improvement gastroscopically when treated with choline chloride [338]. A deficiency of inositol in mice and rats is stated to cause alopecia [188], although a deficiency of pantothenic acid appears to be necessary as well. The alopecia produced in rats fed succinylsulphathiazole (sulphasuxidine), a sulphonamide that inhibits the growth of intestinal flora responsible for the biosynthesis of the B vitamins in the gut, can be prevented by supplements of inositol [339]. Inositol is also stated to be concerned with the regeneration of hair in the condition known as "spectacle eye" in rats [144], although this has not been confirmed, and it is essential for lactation in the rat [150, 232]. The relation of these findings to human nutrition is not known, nor are the human requirements of the vitamin known, although the average intake must be greater than that of any other vitamin owing to its presence in such large quantities in plants and cereal grains. The daily intake is probably about 1 gram; certainly the consumption of

1 to 2 grams daily causes no untoward effects [288]. Vorhaus [288] found that given in doses of this order daily it had no beneficial effect on human alopecia, although it was effective in certain cases of pruritus. Inositol inhibits tumour growth [284]. Recent studies have shown that the livers of patients with gastro-intestinal cancer are infiltrated with fat. If these patients are given inositol in doses of 280 mg. before operation the fat concentration of the liver is found to be within normal limits [825]. In the experimental animal inositol has been found to have a lipotropic effect, *i.e.*, it prevents the deposition of fat in the liver and other organs [19].

CHOLINE



The importance of choline as a food factor was first suggested several years ago by Best [81], who showed that in rats the addition of choline to a diet high in fat prevents the deposition of excess fat in the liver. Choline is, therefore, said to be a lipotropic factor; betaine, the anhydride of choline, and the amino-acid methionine also appear to have a lipotropic action. The exact mechanism of the lipotropic action of choline is unknown, but it is assumed that dietary choline increases the phospholipid content of the liver and this promotes the transport of fatty acids, as phospholipids, from the liver to other tissues, or promotes the utilization of fatty acids in the liver itself [250]. A diffuse nodular cirrhosis of the liver occurs in a number of species kept on diets deficient in choline [285-287], and at the same time hæmorrhagic degeneration of the kidneys has been observed, the lesion occurring in the cortex of the organ [82-84, 87]. Not only is choline essential for the metabolism of neutral fat, but also for that of cholesterol [288]. Choline also prevents the formation of fatty livers in rats on diets deficient in vitamin B₁ [289]. This excess fat is due to synthesis from carbohydrate sources.

Choline is able to prevent specific dietary hepatic injury in rats, and protects against liver damage by toxins such as chloroform [240, 241]. A considerable reduction in phagocytic activity has been observed in rats fed diets deficient in choline [845].

Choline deficiency in animals produces a hæmorrhagic tendency. Intra-ocular hæmorrhages have been observed [242]. The similarity between the enlarged hæmorrhagic kidney and ocular hæmorrhages in choline deficient animals and hypertensive retinopathy in man has been pointed out. However, it is unlikely that choline deficiency exists in man, as lecithin, of which choline is a component, is widely distributed in animal and vegetable foods. Egg yolk, nerve tissue, liver and other offal, and wheat germ are the best sources, although it is also present in greens and leguminous vegetables.

The fact that dietary cirrhosis of the liver in experimental animals can be prevented by choline has led to some speculation on the ætiology and treatment of clinical cirrhosis of the liver. A low intake of protein and the vitamin B complex (including choline) occurs in alcoholics, and it is as unreasonable to assume that alcohol has a directly toxic action on the liver as it is to assume that it affects the nervous or cardiovascular system

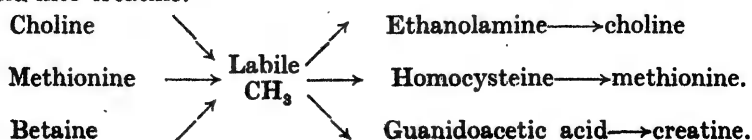
in alcoholic pellagra or beriberi. Favourable results were reported by Patek [248] and Patek and Post [244] in the treatment of clinical hepatic cirrhosis by means of a highly nutritious diet supplemented by the vitamin B complex. In their series, after the onset of ascites seventy-two per cent. of the treated in comparison with fifty per cent. of the untreated series survived six months, and at the end of two years the survival percentages were respectively forty-five and twenty-one. Lowry and his co-workers [245] produced cirrhosis of the liver in rats resembling Laennec's cirrhosis in man. Choline both prevented and cured the condition, although as would be expected, the fibrous tissue persisted. The liver cells regenerated and the gross appearance of the liver improved. Similar findings are reported by Fouts [246]. Milk is not a very good source of choline (1.07 mg. per gram), and the suggestion of Rao [247] that the infantile cirrhosis seen in Hindu children (a variety of portal cirrhosis) may be caused by a diet of cows' milk and a *B. coli* infection merits consideration. Recent clinical studies also point out the importance of a high protein diet in the treatment of patients with hepatic lesions.

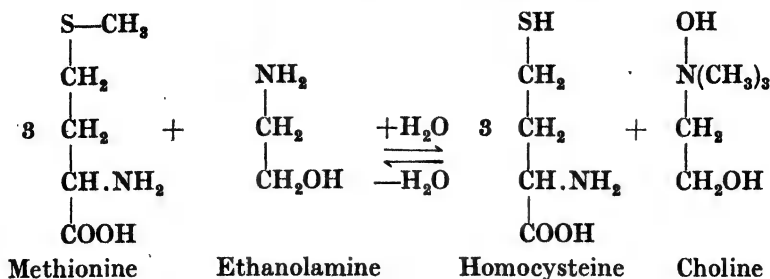
Choline has been used in the treatment of two cases of icterus gravis neonatorum [251]. In a dosage of 5 grams choline daily it was claimed that recovery was accelerated. It was considered to act by enabling the liver to metabolize the increased quantities of fat, which accumulate in this disease and stop the liver from functioning.

Choline is essential for the normal nutrition of the chick and for egg production [35]; for the prevention of perosis or slipped tendon in some birds [40]; for the lactation and normal nutrition of the rat [36]. Generally speaking the young growing animal needs more than the adult. The choline requirement of the dog is about 35 mg. per kilo of body weight daily [248]; that of the chick is 75 mg. daily [249].

In addition to the above-mentioned functions choline is utilized in the animal organism for the formation of acetylcholine.

The methyl groups of choline and the other lipotropic factors, betaine and methionine play a part in the metabolic process known as trans-methylation, which is concerned with the shifting of specific methyl groups as such from one metabolite to another [252-255]. The hypothesis has been advanced that methyl groups in a utilizable form are indispensable in the diet because the animal organism cannot itself generate the methyl groups for the essential methylations. Thus methionine is essential for the growth of young rats. Growth occurs, however, if they are fed a diet containing choline and homocysteine, because a labile methyl group is supplied by the choline and donated to the homocysteine which is thereby converted to methionine. Conversely, choline can be formed by the methylation of ethanolamine, methionine acting as a methyl donor [255]. Another important physiological methylation is the conversion of guanidoacetic acid into creatine.

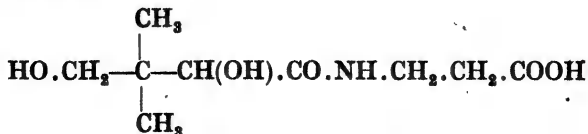




It is possible that future research may reveal that the body can synthesize choline, but until this has been done and its precursors isolated, choline must be considered an essential dietary factor for the animal organism, if not for man.

PANTOTHENIC ACID

History, Isolation and Chemistry. The discovery of pantothenic acid had its origin in 1901 in the discovery of *bios*, a hypothetical substance, now known to be a mixture, necessary for the reproduction of yeast. In 1930 a pellagra-like dermatitis occurring in chicks on restricted diets was described [47], and it was subsequently shown that this can be prevented by feeding pork liver and by a factor in liver extract [48, 49], which was subsequently termed the "filtrate factor." Unfortunately much confusion has resulted because the same term has been applied to the filtrate obtained after the removal of vitamins B₁, B₂ and B₆ from yeast by adsorption. Nutritional achromotrichia (greying of the fur) of rats fed diets deficient in one or more of the B vitamins has been observed by several investigators. Morgan and his co-workers [22] were the first to observe that the active substance which prevents and cures this greying is present in the filtrate factor. The greying of the hair is always symmetrical and is usually observed on the head. A collateral line of research was begun by Williams [256] who found that a naturally occurring compound of unknown composition stimulates the growth of yeast, and to this compound he gave the name "pantothenic acid" on account of its ubiquitous distribution (Greek παντος, everywhere). In 1939 Williams [50] isolated the compound and with Major [51] determined its structure in the following year. It was found to be α -hydroxy- β - β -dimethyl-butryl- β -alanine.



The identity of the chick antidermatitis factor and pantothenic acid, which had been suggested because of their similarity in chemical behaviour [53], was confirmed biologically by Jukes and others [55, 56]. In 1940 pantothenic acid was synthesized by several groups of investigators in

America, Switzerland and Germany [257-259]. Macrae and his fellow-workers [20] showed that the filtrate factor contains pantothenic acid, and subsequently the latter was shown to be identical with the anti-grey hair factor of former investigators [91, 133]. According to Emerson and Evans [155] pantothenic acid does not completely replace "liver filtrate" in the cure of nutritional achromotrichia. Pantothenic acid shows both acidic and basic properties. It is readily soluble in water and acetic acid, slightly soluble in ether, but almost insoluble in the other fat solvents (benzene, chloroform). In a pure condition it is a yellow viscous oil. It is sensitive to heat and to changes in the *pH* of the medium. Pantothenic acid forms a well-defined calcium salt.

Distribution. Pantothenic acid is widely distributed in foodstuffs. Yeast, liver, kidney, wheat bran, and peas are the best common sources, although royal jelly contains about six times as much as yeast [260]. Pantothenic acid is synthesized by certain moulds, bacteria and green plants, and it also appears to be synthesized in the gut of ruminants [141]. Grains are good sources of pantothenic acid, but in the case of wheat at any rate about fifty per cent. is removed in the milling process. A considerable increase in the pantothenic acid content of cereals occurs on germination. About eighty per cent. of the pantothenic acid in foodstuffs is in the bound condition, from which it can be liberated by enzymic digestion with diastase and papain [850].

The distribution of pantothenic acid in various foodstuffs has been determined by Waisman, Mickelsen and Elvehjem [59], using a biological technique. The stewing of meat reduces the pantothenic acid potency to one-third, probably through loss in the cooking-water. In the cooking, dehydration and curing of meat about three-quarters of the pantothenic acid is retained [355]. More recently Snell, Williams [60] and their co-workers have elaborated a biological method of assay depending on the growth response of *Lactobacillus casei*. A method depending on the growth rate of *Proteus morgagni* has also been devised [67].

Pantothenic Acid Content of Foodstuffs [5,137]

	Micrograms per gm.
Apples	0.60
Apricots (canned)	0.95
Asparagus	0.9
Bananas	1.8
Barley	10
Beans, dried	8.8
baked (canned)	1.0
Beef, muscle	4.9
liver	76
heart	20
brain	18
Beets	1.1
Bread, whole wheat	5.7
white	4.6
Broccoli	14
Cabbage	1.8

	Micrograms per gni.
Carrot	2.5
Cauliflower	9.2
Cheese	1.3-9.6
Chicken	5.3-6.2
Chocolate	1.9
Eggs	8.48.
Grape-fruit	2.9
canned	1.2
Halibut	1.5
Lamb, leg	6.0
Lettuce	1.1
Maize	3.1
Milk	2.9
Molasses	2.6
Mushrooms	17
Mutton, shoulder	4.8
Oats	11
Onions	1.3
Oranges	3.4
Oyster	4.9
Peaches (canned)	0.41
Peas, fresh	3.8-10.4
dried	21
Peanuts, roasted	25
Pineapple (canned)	0.06-0.30
Pork, muscle	4.7-11
bacon	2.8-9.8
ham	3.4-6.6
Potatoes	3.2-6.5
Prunes	0.6
Raisins	0.9
"Royal jelly"	89; 511
Salmon	6.6-10
Sardines (canned)	1.0-2.3
Soya bean	18
Spinach	1.8
Strawberries	2.6
Tomatoes	3.7
(canned)	2.5
Turnips	0.37
Veal	1.1-2.6
Wheat, whole	5.1
germ	8.5
bran	20-30. Av. 24
flour, white	3.0
Yeast	140-350. Av. 200

Physiology. Green plants synthesize pantothenic acid after photosynthesis is first established. Yeast normally utilizes pantothenic acid from nutrient media, although it synthesizes small amounts. Some micro-organisms, *e.g.*, lactic acid bacteria and streptococci [261], are unable to

synthesize the substance and must be supplied with the entire molecule, while others synthesize it from β -alanine. There is some evidence that ruminants synthesize pantothenic acid in the gut [141].

Pantothenic acid is essential for the nutrition of many species of animals. It is not known whether it is essential for human nutrition, although it is assumed to be, since the blood pantothenic acid level of patients suffering from deficiency diseases such as pellagra and beriberi is from twenty-three to fifty per cent. lower than in normal persons, and with a rise in the blood pantothenic acid there is an increase of twenty to thirty per cent. in the riboflavin content of the blood [46, 60, 61]. The vitamin is presumably absorbed from the gastro-intestinal tract, and freed from the bound form in which it occurs in food before absorption. The pantothenic acid content of human blood is from 8 to 19.4 μ g per 100 c.c., and that of urine 1.46 to 6.79 mg. in a twenty-four hour specimen [158, 159]. Excretion studies and the results of animal experiments suggest that if pantothenic acid is essential for human nutrition the daily requirements are of the order of 5 mg. to 11 mg. Analysis of human diets, however, reveals that the daily intake on a good mixed diet is much lower than this, *i.e.*, 4 mg. to 5 mg. daily [380]. Pantothenic acid is excreted in human sweat. According to Cornbleet [262] the concentration is 800 μ g per litre; another estimate is 24 μ g excreted in sweat per hour [263]. In human beings pantothenic acid appears to be linked with riboflavin metabolism; thus patients with a low level of pantothenic acid in the blood excrete subnormal quantities of riboflavin. Pantothenic acid is partly destroyed in the body, that escaping destruction being excreted in the urine or stored [264]. In animals on a pantothenic acid-deficient diet, the pantothenic acid content of the tissues is much lower than normal [265].

There is some evidence that pantothenic acid may be concerned with fat and carbohydrate metabolism. Thus young dogs on diets deficient in pantothenic acid develop fatty livers and show lowered values for cholesterol and total lipoids in the blood; feeding pantothenic acid raises the blood lipoids in such dogs [266]. A decreased rate of pyruvate oxidation has also been observed in the liver from rats deficient in pantothenic acid [267]. This suggests indirectly that pantothenic acid may be a component of enzyme systems concerned with the metabolism of pyruvic acid, *i.e.*, of carbohydrate [278]. An increased work output occurs in isolated muscle perfused with fluid containing calcium pantothenate [367].

By excluding pantothenic acid from the diet it has been shown that it is needed by a number of species, including rats, mice, chicks, hogs and dogs. Requirements are increased in experimental hyperthyroidism [162]. Among the effects attributed to pantothenic acid deficiency are nutritional achromotrichia (p. 115), dermatitis, ulcers in the intestinal tract, adrenal, renal and testicular lesions and nerve degeneration. Chicks on a diet deficient in pantothenic acid develop a specific dermatitis, the skin becoming keratinized, and degeneration of fibres in all segments of the cord down to the lumbar region occurs [268, 269]. The vitamin is also essential for the hatching of eggs [270]. If it is withdrawn from the diet the percentage of hatchings is reduced from seventy to three. That natural pantothenic acid deficiencies may exist is shown by the fact that chicks fed on a diet

containing seventy-five per cent. whole corn, supplemented by casein and other vitamins, develop symptoms of pantothenic acid deficiency very rapidly.

Rats maintained on a diet devoid of pantothenic acid cease to grow in three to four weeks and develop a deficiency syndrome characterized by scant, coarse fur, inflammation of the nose and blood-cked whiskers [181]. The so-called blood is in reality porphyrin [156]. A marked weight response in deficient rats is produced by 800 μ g of pantothenic acid or by 50 μ g daily. The maintenance dose for optimum growth is approximately 80 μ g to 100 μ g of pantothenic acid per rat per day [154]. Hæmorrhage, atrophy and necrosis of the adrenals and testicular degeneration have also been described in deficient rats [58]. Such lesions can be prevented or cured by the daily administration of synthetic pantothenic acid for six to fourteen days [124, 125]. A further syndrome in mice fed on diets deficient in pantothenic acid has been described, consisting of loss of weight, paralysis of the hind legs and posterior third of the body, a hyperkeratotic, atrophic and desquamative dermatosis, and myelin degeneration of the sciatic nerves and spinal cord [150].

Dogs kept on a diet deficient in pantothenic acid cease to grow in three to four weeks. Their respiration and pulse rate become very rapid and at necropsy they show fatty degeneration of the liver, hæmorrhagic degeneration of the kidneys, gastritis and enteritis [188]. The pig on a pantothenic acid-deficient diet develops a "goose-stepping" syndrome, which is characteristic, and the gastro-intestinal tract is ulcerated from one end to the other, with the result that the animal becomes emaciated [271]. It also develops a dry, scaly dermatitis. The decreased gastro-intestinal motility seen in some animals on diets deficient in the vitamin B complex may be due in part at any rate to pantothenic acid deficiency. Thus dogs kept on such a deficient diet show decreased gastro-intestinal motility and a decrease in the rates of carbohydrate and protein digestion and absorption [281]. If calcium pantothenate is added there is an immediate return of normal gastro-intestinal function. Inositol also has an effect. Martin and his colleagues [151] have shown that dogs on diets deficient in pantothenic acid and inositol develop an abnormal gastro-intestinal picture, characterized by increased gastric-emptying time, pylorospasm, segmentation of the large and small intestine, hypertonicity and hypomotility (p. 112). The possibility that pantothenic acid metabolism is linked with that of inositol is suggested by Woolley's observation that mice fed on a diet low in pantothenic acid develop symptoms of inositol deficiency. Folic acid and biotin may be essential for the utilization of pantothenic acid [227].

Pantothenic acid deficiency in the experimental animal reduces considerably the phagocytic power of the blood [345] (see also vitamins B₁, B₆, and riboflavin, pp. 108, 172, 320).

Antibacterial Action of Pantothenic Analogues. It is now established that the therapeutic activity of sulphanilamide and its derivatives is due to the fact that they prevent pathogenic organisms from utilizing *p*-aminobenzoic acid, which is a growth factor for these organisms. In

effect a counterfeit growth factor is offered to them, which they accept, but find useless. Sulphanilamide and *p*-aminobenzoic acid resemble one another very closely chemically. Evidence is accumulating to show that other biologically important compounds with similar chemical configurations may interfere with each other with respect to their effects on living cells (p. 212). Many bacteria need small amounts of vitamins, including pantothenic acid, for growth; many pathogenic organisms obtain these from their hosts. McIlwain [271] discovered that pantoyltaurine, $(\text{CH}_3)_2\text{CH}_2\text{OH}.\text{C}(\text{CHOH}).\text{CO}.\text{NH}.\text{CH}_2.\text{CH}_2.\text{SO}_2\text{OH}$, inhibits the utilization of pantothenic acid by pathogenic organisms, such as *Streptococcus haemolyticus*, the pneumococcus, and *Corynebacterium diphtheriae*, in much the same way as sulphanilamide "blocks" *p*-aminobenzoic acid. Such antibacterial compounds in effect starve the micro-organisms of substances essential for their growth. Sulphonamide-resistant streptococci are also sensitive to pantoyltaurine, which may possibly have a practical application in the treatment of infections due to sulphonamide resistant strains of organisms. Its manufacture is relatively easy, although it does not possess the potency of penicillin. Other pantothenic acid analogues with an antibacterial action have been discovered [272]. It is of interest that the syndrome of pantothenic acid deficiency can be produced in mice by feeding pantoyltaurine [273] and also by administering sulphapyridine [274], which not only "blocks" *p*-aminobenzoic acid and pantothenic acid in bacterial nutrition, but prevents its utilisation by the animal. The administration of sufficient pantothenic acid causes the deficiency symptoms to disappear. Unna [328], however, has been unable to produce the syndrome of pantothenic acid deficiency in rats by feeding pantoyltaurine.

Pantothenic Acid in Human Nutrition. Clinical Studies. The rôle of pantothenic acid in human nutrition is unknown. If it is an essential factor for man it would appear from animal data and excretion figures that the daily requirements are of the order of 5 to 10 mg. daily. It is present in human blood (3 to $19.4\mu\text{g}$ per 100 c.c.) and urine (1.46 to 6.79 gm. per twenty-four hours excretion) [158, 159]. A low blood level is associated with a low urinary excretion.

The toxicity of pantothenic acid is extremely low [136, 157]. Thus dogs fed for over a hundred days with 50 mg. a day failed to show any toxic symptoms, and no chronic toxicity was observed in rats fed with 50 to 200 mg. of the substance per day over a long period. 1 gram per kilogram of body weight is tolerated by dogs and monkeys. The daily administration of calcium pantothenate over a period of six months to monkeys (1 gm.), dogs (50 mgm./kilo) and rats (50 to 200 mgm.) did not produce any toxic effects or pathological changes, nor did it influence the metabolism, circulatory and respiratory systems and smooth muscle organs of the animals. Spies [46, 61] has given it in 100 mg. doses intravenously to a number of normal persons without producing any reactions or causing significant changes in blood pressure, pulse or respiration. The pantothenic acid content of the blood and urine is increased soon after the injection, but returns to its previous level within twenty-four hours.

When four persons with cheilosis and typical ocular manifestations diagnostic of riboflavin deficiency received 20 mg. of calcium pantothenate daily for four days, similar temporary increases in blood pantothenic acid and riboflavin occurred, the values returning to their previous levels when treatment was stopped. Spies [61] has also observed that the injection of 200 μ g riboflavin per kilogram of body weight causes an increase of eighty per cent. in the blood flavin concentration and of forty-five per cent. in the pantothenic acid level. These values remained high for three to four hours, but returned to the former level in each instance by the next day.

The production of nutritional achromotrichia in animals by dietary means and the restoration of colour by adding pantothenic acid to the diet (p. 116) led to the hope that it might be effective in the treatment of human grey hair. This was based on a false assumption, because it has never been shown that dietary deficiency is a factor in human greyness. In America widespread publicity was given to the so-called anti-grey hair factors of the vitamin B complex and newspaper advertising was employed to sell calcium pantothenate to restore the colour of grey hair. Several investigations have shown that pantothenic acid or its calcium salt are quite useless for this purpose. Vorhaus [275] used doses of 100 mg. to 500 mg. twice weekly for six weeks without effect. Brandaleone, Main and Steele [276, 366] treated a group of nineteen patients with 100 mg. of calcium pantothenate, 200 mg. of *p*-aminobenzoic acid and 50 grams of brewers' yeast daily for eight months. Changes in colour of the hair were judged by photographs and hair clippings before and after treatment. In only two patients was there an unequivocal change of colour after several months' treatment; in the other subjects a yellow or greenish cast appeared in the grey hair. Kerlan and Herwick [277] also carried out a trial with twenty-six patients, ranging in age from thirty-four to sixty-two years. They were given 20 mg. of calcium pantothenate daily for six months; none reported a significant colour change. A control group not receiving calcium pantothenate remained unchanged. It would thus appear that there is no controlled clinical evidence to show that pantothenic acid is effective in the treatment of grey hair in human beings.

BIOTIN

(Vitamin H, Coenzyme R)

History, Isolation and Chemistry. The discovery of biotin, also known as vitamin H and coenzyme R had its origin in the casual observation of Bateman [279] in 1916 that a high concentration of egg white in experimental diets is toxic; some ten years later certain foods, notably liver and yeast, were discovered which contained an organic substance capable of protecting rats against the toxic effects of egg white, or "egg white injury" [280]. The protective factor was called "the protective factor against egg white injury" and also "vitamin H" by György [10]. It was extensively studied by György and his co-workers [10-16]. In another

laboratory attempts were being made to resolve bios (p. 115), a yeast growth factor, and this resulted in the isolation in 1936 of a crystalline substance from egg yolk by Kögl and Tönnis [281], who named it biotin. About a milligram of active material was obtained from a quarter of a ton of dried egg yolk. Independently in yet another laboratory attempts were being made to isolate a compound, essential for the growth of *Rhizobium*, a nitrogen-fixing organism; this was called "coenzyme R" [282]. It was not realized by these various laboratory groups that they were dealing with the same entity. In 1940 György and his associates [16] announced the identity of biotin with vitamin H and coenzyme R. Biotin was

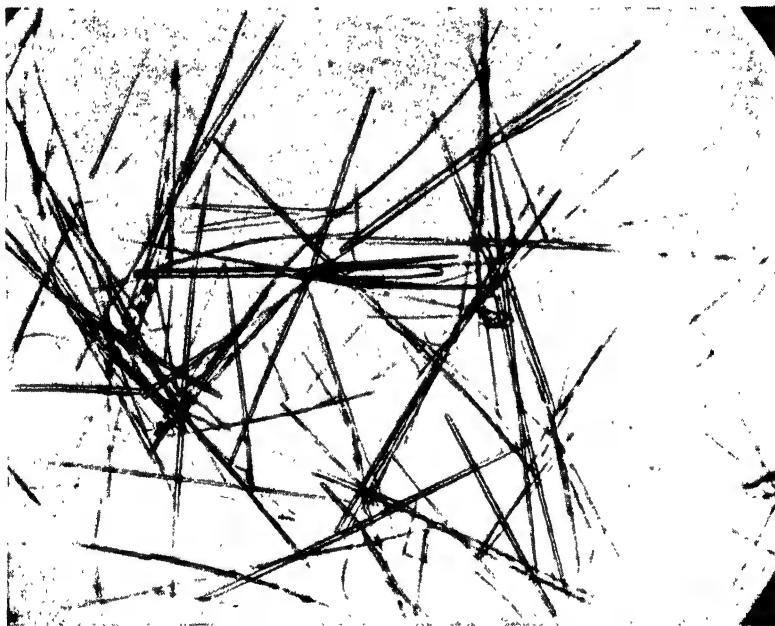
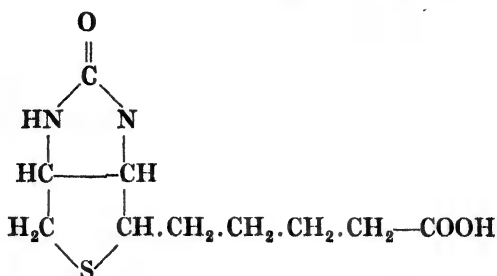


FIG. 25. Crystals of biotin. Magnification $\times 150 \times$.

subsequently isolated from liver [146]. Analytical work by Du Vigneaud and his co-workers [147] in 1941 established the empirical formula, $C_{10}H_{16}O_3N_2S$, and in 1942 they established the structure [283]. The synthesis of biotin was finally achieved in 1948 by Harris and his colleagues [284] in the Merck laboratories in the United States.

Biotin is water- and alcohol-soluble, but relatively insoluble in the fat solvents. It crystallizes in long thin needles (Fig. 25), melting at 280° to 282° C. with decomposition. It is heat stable, and not decomposed by acids or alkalis. The titration curve corresponds to that of a simple monocarboxylic acid. Biotin is a bicyclic urea derivative containing sulphur in a thiophene ring. It is 2' keto-8 : 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid,



A series of papers has recently been published by Kögl and his co-workers which state that the biotin isolated from egg yolk is not the same as that isolated from liver [362]. The two compounds, which differ in physical and chemical properties, have been designated α -biotin and β -biotin, respectively. Neither has been synthesized and neither formula suggested by Kögl agrees with the above formula of biotin put forward by the American workers.

No purely chemical methods are known for the estimation of biotin. The bioassay, based on the cure of egg white injury in rats and chicks [285, 286] is time consuming, and has been replaced by microbiological methods depending on the growth response of yeast [287], *Lactobacillus* [288] and other organisms [289]. A method depending on the titratable acidity produced by *Lactobacillus casei* has been developed [290].

Units and Distribution. Until the isolation and identification of biotin biological units were employed to denote the potency of biotin-containing material. The "rat unit" of biotin is the daily dose of a standard preparation which will cure egg white injury produced in rats on a special diet [285]; this corresponds to $0.1\mu\text{g}$ of biotin or 10,000 units per milligram of biotin [298]. The "yeast growth" unit [281] is the amount of biotin producing a one hundred per cent. increase in cell growth of a specified strain of yeast under certain conditions; this corresponds to 27,000 yeast growth units per milligram [294].

Biotin is found in foodstuffs containing other members of the vitamin B complex, particularly yeast, liver, kidney, and other offal, light chicken meat, eggs and peas, cocoa and cereals. An increase in the biotin content of cereals occurs on germination. Biotin is present in most foodstuffs in a bound form, from which it is liberated in the intestine by enzymic hydrolysis [291]. An average of seventy-seven per cent. of the biotin is retained in meat after cooking [329]. The biotin content of some common foods is given in the following table :—

Biotin Content of Foodstuffs [5]

	Micrograms per gm.
Apples	0.009
Bananas	0.044
Beans, dried	0.098
Beef, muscle	0.026
Beets	0.027

	Micrograms per gm.
Bread, whole wheat	0·019
white	0·011
Cabbage	0·024
Carrot	0·025
Cauliflower	0·17
Cheese	0·086
Chicken	0·054—0·098
Chocolate	0·82
Eggs	0·090
Grape-fruit	0·080
Halibut	0·08
Lamb, leg	0·021
Lettuce	0·081
Maize	0·058
Milk	0·050
Molasses	0·091
Mushrooms	0·16
Mutton, shoulder	0·027
Onions	0·085
Oranges	0·019
Oyster	0·087
Peas, fresh	0·085
dried	0·18
Peanuts, roasted	0·84
Pork, muscle	0·02—0·046
bacon	0·075
ham	0·04—0·06
Potatoes	0·006
Raisins	0·081
“Royal jelly”	1·7
Salmon	0·053
Spinach	0·069
Strawberries	0·040
Tomatoes	0·040
Turnips	0·021
Veal	0·014—0·02
Wheat, whole	0·052—0·07
flour, white	0·007
Yeast (<i>Torula utilis</i>)	1·88
(Brewers')	0·071

Physiology. Biotin is necessary for the growth of many bacteria, *e.g.*, staphylococcus, strains of clostridium, yeasts and fungi [287, 295, 296]. It is also a growth hormone for higher plants, and a growth factor for the rat [300] and most animals. The vitamin appears to be related to the fundamental process of growth since the biotin content of embryo tissues and tumour tissue is very high [148]. The growth of nerve tissue is stimulated three to four times the normal rate in the presence of biotin; cells from the brain, spinal ganglia, skin and mesenchyme also show stimulation of growth [302]. Many bacteria and moulds are able to synthesize biotin [298] and there is evidence that bacterial synthesis

occurs not only in the gut of animals [299], but also in the human gut, as the excretion in the urine and faeces is greater than the intake [301]. Biotin is absorbed from the intestine and excreted in the urine; whether that excreted in the faeces is entirely derived from bacterial synthesis or whether excretion occurs into the gut or some is not absorbed is not known. Some storage occurs in the liver, kidneys, heart and adrenals.

If rats are given purified diets containing sulphaguanidine or succinylsulphathiazole, which are bacteriostatic, signs and symptoms of biotin deficiency are produced, the effect being presumably due to interference with the bacterial synthesis of biotin in the intestine [303]. The deficiency syndrome can be prevented and cured by administering biotin by mouth. *p*-Aminobenzoic acid can also produce biotin deficiency [304]. These observations are of interest because similar interference can be produced in the bacterial synthesis of folic acid (p. 111) and vitamin K (p. 807). In the mouse biotin deficiency causes alopecia [368].

Biotin deficiency also results in a specific dermatitis in chicks [182, 317], affecting the under surface of the feet, which become roughened and calloused and finally crack. Other lesions occur at the corners of the beak and eyelids.

The physiological action of biotin is not known. A decreased rate of pyruvate oxidation occurs in the liver of rats deficient in biotin [267]. This suggests indirectly that biotin may be a component of the enzyme systems concerned with the metabolism of pyruvate and hence carbohydrate (*cf.* pantothenic acid, p. 118). Biotin produces marked change in aerobic glycolysis, resulting in increased production of bicarbonate from increased utilization of lactate [369]. There is also a suggestion that biotin may play a rôle in fat synthesis and metabolism [305].

The sulphur atom of biotin has been replaced by hydrogen, forming a compound known as desthiobiotin [306], which has an anti-biotin action [358]. Several other anti-biotin compounds, including biotin sulphone, have been prepared [359].

Avidin and Egg White Injury. The syndrome produced in rats by feeding large quantities of uncooked egg white, first described in 1927 by Boas [280], is in effect the syndrome of biotin deficiency. The extensive work of Parsons and her associates from 1931 to 1940 demonstrated that there exists in egg white a basic protein that combines with biotin and renders it unavailable to the organism, presumably by preventing its absorption from the gastro-intestinal tract [307]. The growth of organisms known to require biotin can be completely inhibited by raw egg white [308]. The isolation and properties of the protein, which has been called avidin, has been described [149], and its crystallisation and analysis effected [309]. It is a glycoprotein and forms a complex with biotin, which cannot be broken down by proteolytic digestion, but only by heating. This explains why raw egg white inactivates biotin physiologically, but cooked egg white does not. The combination between biotin and avidin is stoichiometric and separation cannot be effected by dialysis. Avidin can replace raw egg white as the toxic factor in egg white injury. There is some evidence that biotin can also be inactivated by rancid fats [310]. It is indeed peculiar that the avidin-biotin complex cannot be broken down by the gastro-

intestinal enzymes, yet it can be utilized if injected [811]. Avidin is one of the first of a class of nutritionally important substances that might be termed anti-vitamins. Other anti-vitamins are the anti-vitamin B₁ factor of raw fish (p. 219) and dicumarol (p. 888).

Biotin deficiency through feeding raw egg white or diets deficient in biotin has been produced in rats, chicks, monkeys, dogs, and even human beings [175, 812-814]. The syndrome has been most extensively studied in the rat, in which it is characterized by a generalized erythematous,



FIG. 26. Biotin Deficiency in a Rat. This animal, age eighty-five days, has received a diet containing thirty-five per cent. uncooked egg white since the age of twenty-one days, and exhibits typical acute, exfoliative, desquamative dermatitis, accompanied by loss of hair and abnormal posture. The dermatitis is particularly severe about the flanks, fore feet and head.

scaly, greasy pruritic dermatitis, arrest of growth, an abnormal posture and spastic gait [280, 285, 815]. This is shown in Fig. 26. A typical atrophy of the fur around the eyes occurs, giving the condition known as "spectacle eye." These changes can be prevented or cured by the administration of biotin concentrates, pure biotin, or its methyl ester. Degenerative pathological changes have also been described in the thymus, testes and epididymis, and the muscle tissue is pale and atrophied [816]. György has pointed out the similarity between the cutaneous lesions of biotin deficiency in the rat and seborrhœic desquamative dermatitis in man. Daft and his colleagues [821] have induced biotin deficiency in rats

on purified diets by feeding sulphaguanidine, succinylsulphathiazole and liver extracts in place of egg white. They have observed not only a typical dermatitis, but a granulocytopenia, leukopenia and anæmia; hyalinization and necrosis of voluntary muscle; and hyaline sclerosis and calcification of blood vessels.

Cattle do not necessarily need an exogenous supply of biotin, as this is synthesized by bacteria in the rumen [319]. The pig is able to thrive on a ration containing 5 lbs. of raw egg white, suggesting either that this animal does not need biotin, or else its digestive juices can break down the biotin-avidin complex [318].

Biotin may be associated with certain immunity phenomena since it is known to activate lysozyme, the lytic enzyme in tears, mucus, sputum and body fluids that digests bacteria [343]. The biotin-avidin complex is also bacteriolytic, being only "toxic" in the sense that it inactivates the essential metabolite biotin [344].

Clinical Studies on Biotin. The urinary excretion of biotin in man on unrestricted diets varies from $7\mu\text{g}$ to $89\mu\text{g}$ per litre or $14\mu\text{g}$ to $111\mu\text{g}$ in a twenty-four-hour specimen of urine. The excretion of biotin varies daily and throughout the day [320]. It is not influenced by disease, and even during periods of starvation the excretion is not abnormally low. If the daily intake is constant the urinary excretion is constant. There is an increase after the ingestion of biotin or liver. The average diet supplies $30\mu\text{g}$ to $40\mu\text{g}$ of biotin daily; this is increased to $65\mu\text{g}$ if liver is added to the diet. Biotin is also excreted in the fæces, and since the biotin content of the latter is greater than the intake, synthesis of biotin must occur in the human body, presumably in the intestine through the agency of bacteria. (cf. p. 186). Small amounts of biotin are excreted by human beings on diets containing large amounts of egg white [320].

There is good evidence that biotin is essential for human nutrition. Sydenstricker and his colleagues [175] have reported a deficiency syndrome in four volunteers kept on a diet poor in biotin, lack of which was accentuated by including large quantities of egg white in the diet. Vitamin supplements were added so that the diet was adequate in all other respects. At the beginning of the experiment all the volunteers were in good condition and free from symptoms and signs of avitaminosis. During the third and fourth weeks all developed a fine, scaly dermatitis, which disappeared spontaneously. After the seventh week one volunteer developed a maculosquamous dermatitis of the hands, arms and legs. During the seventh and eighth week all showed a striking greyish pallor of the skin, which was interpreted as a sign of vasoconstriction. Eventually all the volunteers developed a definite atrophy of the lingual papillæ, which was either general or patchy with the production of a "geographical" tongue. The tongue remained pale, and in no way resembled that seen in ariboflavinosis (p. 385) or pellagra (p. 403). By the ninth and tenth week all showed dryness of the skin of the extremities, with a tendency to fine branny desquamation. After the fifth week many of the symptoms associated with vitamin B₁ deficiency (p. 227) began to appear—mild depression to extreme lassitude, muscle pains, hyperæsthesia, localized paræsthesiæ, anorexia and nausea. Blood examinations showed a fall in



FIG. 27. Section of Skin from Case of Human Biotin Deficiency. It shows loose sheets of keratinized epithelium, absence of rete pegs and cellular infiltration around the hair follicles, sweat glands and blood vessels. The corium is thickened, hair follicles atrophic and sebaceous glands absent. See text, p. 129.

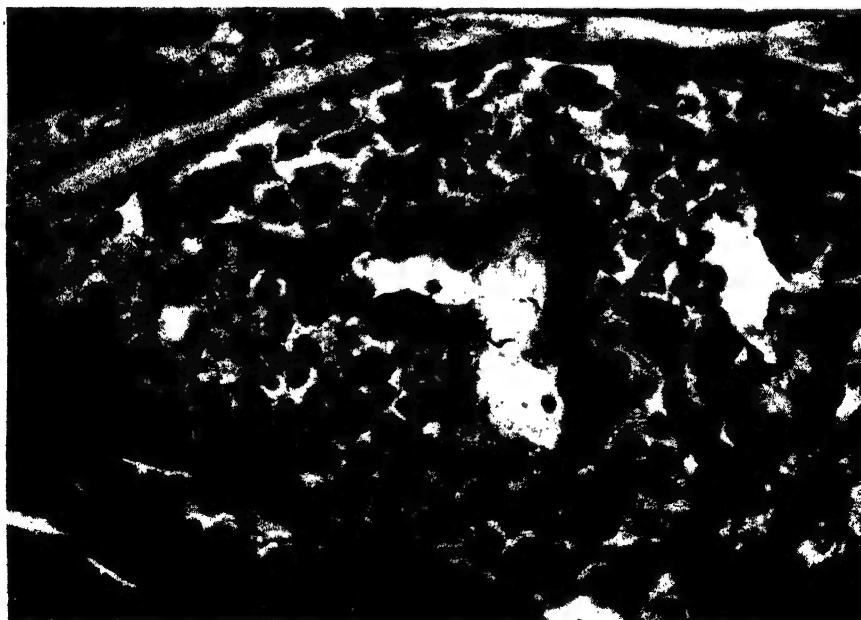


FIG. 28. Section of Skin from Case of Human Biotin Deficiency. It shows a marked cellular reaction around the small blood vessels in the corium. See text, p. 129.

hæmoglobin percentage, the number of erythrocytes and volume of packed red cells; these changes occurred in spite of an adequate iron intake. There was a striking rise of bile pigments and cholesterol in the blood. The urinary excretion of biotin fell to $8.5\mu\text{g}$ to $7.3\mu\text{g}$ in twenty-four hours. Treatment with an injectable biotin concentrate, $75\mu\text{g}$ to $800\mu\text{g}$ daily, resulted in relief of signs and symptoms in three to five days. The minimal amount of biotin for prompt relief was $150\mu\text{g}$ daily. The urinary excretion rose from $8\mu\text{g}$ to $7\mu\text{g}$ to an average of $55\mu\text{g}$ of biotin daily.

A case of clinical biotin deficiency has been reported by Williams [321]. The patient was an old retired Italian labourer, who suffered for years from an exfoliative dermatitis and mild conjunctivitis. There was nothing obvious in the history of the case to account for the rash, but the dietary history was significant. Since adolescence the patient had a passion for raw eggs, the consumption of which ran into six dozen weekly. The whites of twelve eggs contain sufficient avidin to bind $280\mu\text{g}$ of biotin [322]. Therefore, it would seem that little if any biotin would be absorbed from the gastro-intestinal tract. His choice of foods was narrow, and excluded good sources of biotin; the diet included one to four quarts of wine daily. The skin lesion, which was studied by biopsy, did not correspond to that due to a deficiency of nicotinic acid, riboflavin, pantothenic acid or vitamin B₆, but closely resembled that seen in animals with biotin deficiency. Half the total surface of the body appeared normal, but the entire face, ears, shoulders, dorsum of forearms, hands and lower legs were a fiery red and covered with scales. Skin biopsy showed loose sheets of keratinized epithelium, flattened rete pegs, and cellular infiltration around the hair follicles, sweat glands and blood vessels (Figs. 27 and 28). The epidermis showed hyperkeratinization, and parakeratinization, and the corium was thickened, due to interstitial oedema and hypertrophy of the collagen fibres. Sebaceous glands were absent, hair follicles atrophic, and sweat glands and ducts dilated. The macroscopic and microscopic changes of the skin were compatible with a diagnosis of biotin deficiency, although not pathognomonic of the condition as seen in animals. Before treatment the serum biotin was low. After the patient had been hospitalized for a fortnight on a liberal diet and given injections of biotin methyl ester, the dermatitis largely disappeared, the serum biotin returned to normal levels, and the patient's general condition improved. This would appear to be the first recorded case of clinical biotin deficiency.

As far back as 1929 Findlay and Stern [154] suggested a similarity between the symptoms of egg white injury in the rat and those in children with acrodynia (pink disease). So far no clinical studies have been made with biotin in acrodynia. Marshall [78] claims to have obtained curative results with biotin concentrates in the treatment of acne vulgaris, rosacea and furunculosis.

It has been suggested that there is some indirect relationship between biotin and carcinogenesis. If butter yellow (*p*-dimethylaminoazobenzene) is fed to rats hepatic tumours are produced, although their formation can be prevented by protective diets rich in protein and the vitamin B complex, particularly riboflavin (p. 320). This protective effect is broken down by

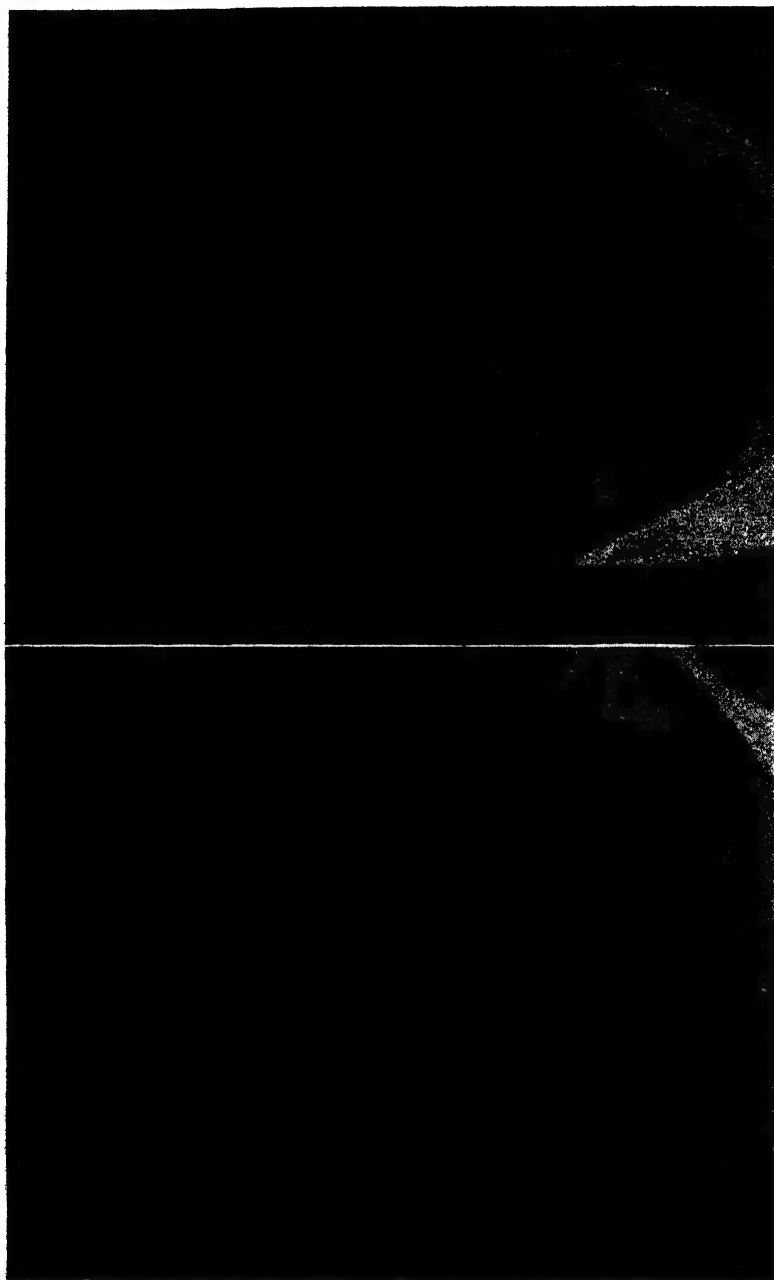


FIG. 29. A Case of Vitamin B Deficiency in a London Woman before Treatment. Presenting complaints : depression ; insomnia ; memory impairment ; red cracked lips ; glossitis ; dermatitis on exposed parts, flexures and vulva ; photo-

FIG. 30. Same case as Fig. 29 after several weeks' treatment with a yeast concentrate, vitamin B₁, riboflavin, nicotinic acid and improved diet.

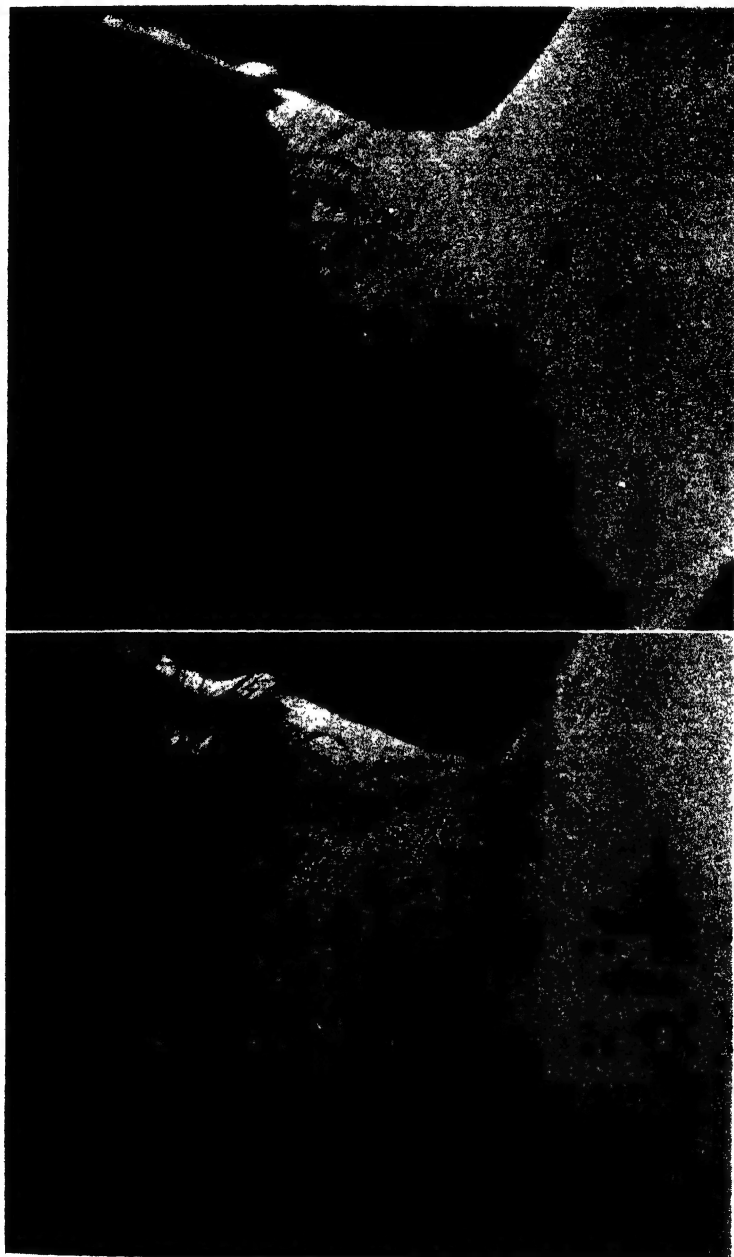


FIG. 31. A Case of Vitamin B Deficiency in a London Woman before Treatment. Same case as Fig. 29, showing scaly pellagroid dermatitis at back of neck.

FIG. 32. Same case as Figs. 29 to 31. After treatment with a yeast concentrate, vitamin B₁, riboflavin, nicotinic acid and improved diet.

the addition of biotin to the diet [44]. Tumour tissue, including carcinoma, also contains abnormally high concentrations of biotin [289]. It is, therefore, reasonable to assume that biotin may be essential for the growth of neoplastic tissue. If this be so the withholding of biotin from patients with cancer might decrease the growth of neoplastic tissue to a greater extent than that of normal tissue. To test this Rhoads and Abels [828] attempted to induce biotin deficiency by administering egg white in two patients with cancer. They were given 375 gm. of raw frozen egg white and 165 gm. of dried egg white daily for thirty weeks, a quantity supplying sixteen to forty times the avidin necessary to bind the biotin in their diets. No clinical evidence of biotin deficiency was observed in the patients, nor was the biotin excretion abnormally low. There was no improvement in the clinical condition of either patient. One died thirty-four weeks after the commencement of the experiment; the other eventually improved after a course of X-ray therapy. To be satisfied that the administration of egg white has no effect on the growth of cancer tissue, it would be necessary to establish clinical and experimental evidence of biotin deficiency in the patients. Rhoads and Abel also found that the growth of spontaneous mammary cancer in mice was not affected when a typical biotin deficiency was induced. This has been confirmed [297]. Moreover, the inoculation of fragments of sarcoma 37 or sarcoma 180 into mice already suffering from biotin deficiency is followed by a tumour growth, appearing at the expected rapid rate [39].

Kaplan [333] treated a group of ten cancer patients with egg white, corresponding to 2,000 units of avidin daily, but was not able to observe a definite cure over a period of a year. Viewing the cases as a whole, he considered that the general condition was markedly improved; there was less cachexia and the appetite was improved.

p-AMINO BENZOIC ACID

Ansbacher [134] has shown that *p*-aminobenzoic acid is a vitamin, since it is necessary for the normal pigmentation of the rat and is a growth-promoting factor for the chick. It is also a factor in lactation in the rat [38]. The vitamin is widely distributed over the entire plant and animal kingdom. It is an essential metabolite for bacteria [135]. The bacteriostatic action of the sulphonamides is considered to depend upon the close similarity in structure between the latter, which are derivatives of sulphanilamide



[25, 135]. It is believed that the sulphonamides are taken up by the bacterial enzyme system which normally uses *p*-aminobenzoic acid; the enzyme system then becomes blocked and incapable of normal function, with resulting interference in the reproduction of the bacteria, which then become a prey to the defence mechanisms of the body. The enzyme system has a greater affinity for *p*-aminobenzoic acid than for the sulphonamides, which to be effective must be in much greater concentration. Thus 1,600 parts of sulphanilamide are necessary to compete with one part

of *p*-aminobenzoic acid. Conversely, *p*-aminobenzoic acid in sufficient concentration can annul the bacteriostatic effect of the sulphonamides *in vitro* and *in vivo*. Sulphanilamide can replace *p*-aminobenzoic acid in a synthetic diet for the rat, preventing deficiency symptoms which normally result if *p*-aminobenzoic acid is omitted from the diet [24]. It has been claimed that *p*-aminobenzoic acid diminishes the toxicity of acetarsone, neoarsphenamine, tryparsamide and related arsenical drugs [324]. Clinically it has been found that *p*-aminobenzoic acid does not prevent nitritoid crises, gastro-intestinal reactions or optic nerve injury produced by tryparsamide [352]. The protective action is not specific, as it is also exerted



FIG. 33. A Case of Vitamin B Deficiency in a London Woman before Treatment. The photograph shows angular stomatitis and a red fissured tongue.



FIG. 34. Same case as in Fig. 33 after treatment with vitamin B₁, riboflavin, nicotinic acid and wheat germ and yeast preparations.

by other related organic acids, such as benzoic, phenylacetic and phenylpropionic acids [351].

Clinical interest has centred around *p*-aminobenzoic acid because of its supposed relation to grey hair. Deficiency in the black or piebald rat results in a greying of the fur [21, 134]. A rather premature claim was made in 1941 by Sieve [152] that *p*-aminobenzoic acid is effective in the treatment of human grey hair, particularly in younger middle aged persons, in whom the greyness was premature. Unfortunately much publicity was given to anti-grey hair factors in the American lay press. In a trial carried out by Brandeleone, Main and Steele [276, 366] it was found that a daily dose of 200 mg. of *p*-aminobenzoic acid given for several months had no significant effect upon the colour of grey hair in human beings (p. 121). Eller

and Diaz [326] treated eighty-eight grey-haired patients with 100 mg. of *p*-aminobenzoic acid three to four times daily for three to five months. In not a single case was there a change from white or grey to normal; a recordable change in colour was noted in four patients. Whether this was due to the treatment or to a general improvement in health is unknown. A possibility that cannot be excluded is the excretion into the hair of a *p*-phenylenediamine-like compound, derived from *p*-aminobenzoic acid, acting as a dye. In another group of two hundred patients treated with a vitamin B complex mixture derived from brewers' yeast some (number unspecified) are reported to have shown darkening of the hair. A Continental author [23] also claims that his grey hair became darker in colour after taking 5 grams of a vitamin B preparation daily for two years. In conclusion it would appear that there is no evidence that human grey hair is due to a nutritional deficiency, nor is there any controlled evidence that any of the B vitamins can restore the natural colour or darken grey hair. A warning is also necessary against the continuous ingestion of large and unphysiological doses of *p*-aminobenzoic acid. Although the toxicity factor is low, a high blood level of the substance would render sulphonamide therapy ineffective should the patient suffer from an acute infection normally amenable to treatment with sulphonamides.

A premature claim was also put forward for the use of *p*-aminobenzoic acid in the restoration of pigmentation in vitiliginous patches in depigmented areas in patients with alopecia areata [353, 373]. Depigmentation was also stated to occur in areas of hyperpigmentation, such as naevi, naevus cells and lentigines. Further investigations by Beinhauer [354] could not substantiate these claims. Vitiligo remained unchanged in forty-one patients treated with *p*-aminobenzoic acid, and repigmentation occurred in only two out of fourteen patients with achromotrichia; in these it may have been due to hair shampoo. No repigmentation was noted in eleven patients with achromotrichia associated with alopecia areata, and no prevention or depigmentation of lentigenes was observed in six patients under treatment.

Rothman and Rubin [336] have made the interesting observation that ultraviolet erythema is due in part to the photochemical reaction of *p*-aminobenzoic acid in the skin. They state that if incorporated in an ointment to the extent of ten to twenty per cent. it protects against sunburn.

Following the observation that the mortality of experimental murine typhus in white mice was reduced from eighty per cent. to under twenty per cent. by the oral administration of *p*-aminobenzoic acid, trials were made with the substance clinically at the American Typhus Commission Unit at Cairo [363]. The dosage was empirical, the initial dose being from 4 to 8 grams, followed by 2 grams every two hours up to a total dose of from 60 to 216 grams. The dosage was adjusted to give a blood concentration of 10 to 20 mg. per 100 c.c. Twenty cases were treated against forty-four controls and the impression was gained that if *p*-aminobenzoic acid is given in the first week of illness the clinical course of the disease is much less severe than in control patients.

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CHAPTER III

VITAMIN B₁

(ANEURIN, THIAMIN)

HISTORY

THE history of vitamin B₁ is intimately associated with studies on beriberi. It is stated that the disease was known in China and that it was first described clinically by Bontius [1] in 1642 and by Tulp [2] in 1652. The description given by these older writers suggests, however, that the disease was not the same as that we know to-day. Modern beriberi dates from the introduction of steam-powered rice mills in the nineteenth century. When beriberi became prevalent in the East it was confined to those persons whose diets consisted largely of highly milled or polished rice from steam mills. In the same regions, those natives who ate rice ground in primitive mills largely escaped.

The conquest of beriberi starts from 1882, when Takaki [5], the Japanese Director-General of the Medical Department of the Navy, practically eradicated the disease among Japanese sailors by supplementing the diet, which consisted mainly of rice, with fish, vegetables, meat and barley. Whereas between the years 1878 and 1882 the incidence of beriberi in the Japanese Navy ranged from twenty-three to forty per cent. of the ratings, Takaki reduced it by dietetic measures to less than 0.5 per cent. Steps were also taken by the Dutch Government to deal with the disease in the Dutch East Indies, where a group of medical officers, directed by Christiaan Eijkman, initiated investigations which ultimately led to the discovery of vitamin B₁. In 1897 Eijkman [6] showed that a paralytic condition in fowls, *polyneuritis gallinarum*, which closely resembled beriberi in its polyneuritic symptoms, could be produced by a diet consisting of polished rice. When the fowls were fed on unpolished rice they did not develop the disease, which was also cured in afflicted birds by the administration of rice polishings. Eijkman also demonstrated the presence of an anti-neuritic principle in aqueous and alcoholic extracts of rice polishings. Grijns [7] (1901), Eijkman's successor in the East Indies, concluded that both beriberi and avian polyneuritis resulted from the lack of a certain dietary factor or factors present in rice bran. His work focussed attention on beriberi as a deficiency disease, but this view was not seriously considered until Fletcher [8], Fraser and Stanton [9] confirmed it by their studies on beriberi in the Malay States between 1905 and 1910. Fletcher, working in an asylum at Kuala Lumpur, separated the patients into two groups, one of which was supplied with polished rice, the other receiving brown rice. In the first group thirty-six out of 120 developed beriberi, and eighteen died from the disease; in the second group only two out of 128 developed the disease and there were no deaths. Fraser and Stanton took 800 healthy labourers into a railroad labour camp. To half of them the customary polished rice was given as a staple article of

diet, the rest receiving the unpolished grain. In three months time beriberi was rife amongst the white rice group, while those on unpolished rice were practically free from the disease. Later the rice rations were reversed in the two groups, with the result that the disease disappeared in the first group and an epidemic of beriberi broke out in the second.

In 1911 Funk [18], of the Lister Institute, attempted to isolate from rice polishings the principle that was active in curing beriberi and avian polyneuritis, and he succeeded in obtaining a concentrate which was capable of curing polyneuritis in pigeons in doses of 20 mg. From a study of the literature Funk put forward the theory that not only beriberi, but also scurvy, pellagra and possibly rickets were due to the absence of certain specific factors from the diet. He believed that these factors, which he termed "vitamines," were of the nature of organic bases, whence the ending *-amine*. Funk considered that he had isolated the anti-beriberi or antineuritic vitamin in pure form, but subsequent research has shown that his preparation, although rich in vitamin B₁, was not the pure substance.

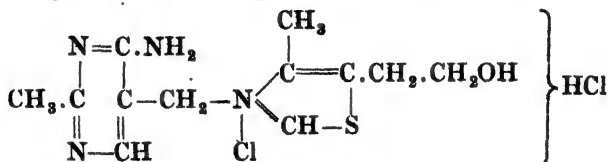
In the meantime Osborne and Mendel (1911, 1918) [10], in America, had shown that butter contained a growth-promoting factor essential for the development of rats. This factor, now known as vitamin A, was discovered independently by McCollum and Davis [11] in 1918 in egg yolk as well as in butter and cod-liver oil. These two investigators further discovered that crude milk sugar used in the rats' diets contained another essential growth-promoting factor, which was also found to be widely distributed in a number of foods, including milk, yeast, rice polishings, and wheat embryo. McCollum and Davis (1915) [12] concluded that: "There are necessary for normal nutrition during growth two classes of unknown accessory substances, one soluble in fats and accompanying them in the process of isolation from certain foodstuffs and the other soluble in water, but not apparently in fats." McCollum called the growth factor present in butter and cod-liver oil "fat-soluble A," owing to its solubility in fat solvents. The other growth factor present in milk, yeast, rice polishings and wheat embryo, was termed "water-soluble B." It was soon realized that "water-soluble B" and the anti-beriberi or anti-neuritic factor had similar properties and distribution, and it was therefore assumed that they were identical. The two systems of nomenclature were therefore combined and the factor renamed *vitamine B*, and in 1920, following a suggestion of Dummond [18], the terminal *e* was omitted.

Many attempts were made to isolate the antineuritic factor from rice polishings and although highly active preparations were obtained by several workers, it was not isolated in a pure form until 1926, when Jansen and Donath [25] succeeded in obtaining 100 mg. from 8 kg. of rice polishings. In that year Smith and Hendrick [15] showed that vitamin B consisted of a heat labile antineuritic component and a heat stable component. These were renamed *vitamin B*₁ and *vitamin B*₂. The resolution of the latter into a number of other components is described on p. 98. In 1935 Jansen [31] suggested for pure vitamin B₁ the name *aneurin*, a word derived from *a*(nti-*poly*)*neur*(itis) *vitam**in*. Williams in America proposed the alternative name *thiamin* on account of the fact that it contains a thiazole grouping; this name is retained in the American literature.

In 1932 Windaus and his collaborators [32] isolated vitamin B₁ from yeast and determined the correct empirical formula of the vitamin. Further work in 1934 by Windaus, Tschesche and Grewe [33] in Germany, and by Williams [34, 38-40] and his school from 1934 to 1936, led to the elucidation of the chemical structure of the vitamin. The final chapter in its history was written by Williams and his co-workers [35], who brought their work to a brilliant conclusion by the synthesis of vitamin B₁ in 1936. Shortly afterwards other alternative syntheses were published by Williams [42], Andersag and Westphal [36] of the I.G. laboratories at Elberfeld, and by Todd and Bergel [37] at Edinburgh. The synthetic vitamin proved to be biologically identical with that obtained from natural sources [43].

CHEMISTRY OF VITAMIN B₁

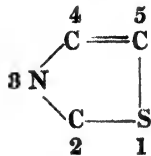
Vitamin B₁ has the following structure :



It is composed of pyrimidine and thiazole nuclei :



Pyrimidine.



Thiazole.

Vitamin B₁, or 2-methyl-5-(4-methyl-5-β-hydroxyethyl-thiazolium chloride) methyl-6-aminopyrimidine hydrochloride, is a colourless crystalline substance containing a molecule of water of crystallisation and melting at 248°-250° C.

Vitamin B₁ is sensitive to ultraviolet light of less than 290 mμ, with maximal photochemical decomposition at 255mμ at pH7. The effect of heat on vitamin B₁ is important on account of the possibility of its destruction in the ordinary processes of cooking and canning. The rate and extent of destruction is markedly increased by the presence of water and a rising pH. In the dry condition it is stable at 100° C. for twenty-four hours, even in contact with air. The effect of heat on vitamin B₁ depends not only on the pH but on the electrolyte system. Thus at pH 5.4 for an hour one hundred per cent. destruction occurs in the presence of borates, fifty-seven per cent. in unbuffered aqueous solution, ten per cent. in acetates, and three per cent. in phosphates [50]. The destruction by heat is a function of time, temperature, pH, and the presence of electrolytes. Meat curing ingredients (sodium chloride, nitrate and nitrite, cane sugar and glucose) have no significant effect in accelerating destruction

during heating [50]. Copper catalyses the rate of destruction of vitamin B₁, but iron, aluminium, zinc and tin do not [80]. This is of some significance as these metals are present in cooking utensils.

Destruction due to heating in the ordinary process of cooking is not very great, provided the cooking temperature is not much above 100° C., the reaction is not alkaline, and the heating is not too prolonged. Considerable inactivation occurs in pressure cookers. The presence of other components in the foodstuff under consideration may facilitate the decomposition of the vitamin. The destructive action of sulphites is of some importance, since these are used in the preservation of fruit pulp and juices [55]. The sensitivity of vitamin B₁ to sulphites, which inactivate it, depends on the pH of the medium. Thus decomposition is instantaneous

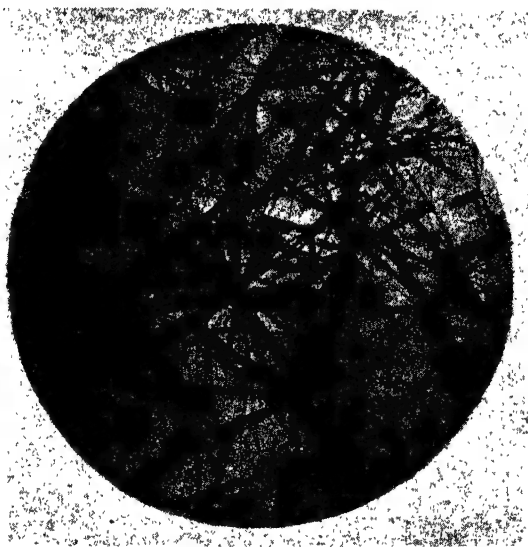


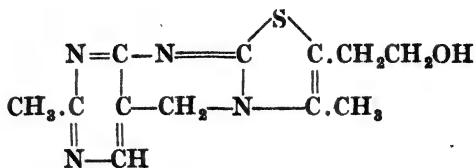
FIG. 35. Crystals of Vitamin B₁ Hydrochloride.

at pH 6; at pH 5 about eight to twelve hours is required for complete decomposition; at pH 3 there is relatively little destruction even after a period of months. Vitamin B₁ is fairly resistant to heat in faintly acid or acid media. Below pH 5.0 aqueous solutions are fairly stable to boiling [80]. In sealed tubes at 100°–125° C., when the pressure is considerable, 0.1 per cent. solutions at pH 3–6 are unaffected for short periods. This is of importance in sterilizing solutions for parenteral injection. Such solutions (pH about 3.5) may be heated for half an hour at 100° C. or twenty minutes at 120° C. with little loss of potency.

The effects of cooking, canning, freezing and dehydration on the vitamin B₁ content of foodstuffs are described on p. 156.

Vitamin B₁ is not oxidized by atmospheric oxygen under ordinary conditions. When subject to mild oxidation with potassium ferricyanide in alkaline solution it is oxidized to thiochrome [44], a compound isolated

from yeast by Kuhn [45], and shown by Todd and his colleagues [46] to have the formula :—



Thiochrome.

Thiochrome shows an intense blue fluorescence under ultra-violet light, a reaction employed in the estimation of vitamin B₁ (p. 234). Thiochrome is devoid of any antineuritic action.

Vitamin B₁ analogues with an antineuritic action are known [29].

UNITS OF VITAMIN B₁

Before the isolation of pure vitamin B₁ the antineuritic potency of a substance was expressed in terms of dry rice polish or dried yeast, which were obviously unsuitable standards on account of their varying activity. To secure uniformity the Health Organization of the League of Nations [419], in 1931, adopted an international standard, consisting of an acid clay adsorbate of vitamin B₁ from rice polish extract, prepared in a standard manner described by Jansen and Donath. An International Unit was defined as the antineuritic activity of 10 mg. of the adsorbate. Difficulty was again experienced because the value of the unit varied with the method of biological assay, the animal used, its age, size, and its general condition. Moreover, there was no evidence that the vitamin B₁ was completely released from the adsorbate in the intestine of the test animal.

Now that pure vitamin B₁ is available it is hoped that the vitamin B₁ content of various materials and the dosage of the vitamin will be expressed in milligrams or micrograms* of crystalline vitamin B₁. An endeavour has been made in this book to drop the use of International Units, except in food tables. The International Unit, however, is still retained in much of the literature, and until 1938 was generally considered to be equivalent to 2 µg of pure vitamin B₁ (*i.e.*, 500 I.U. ≡ 1 mg.). In that year the International Conference on Vitamin Standardization recommended that the International Unit be defined as possessing the antineuritic activity of 3 µg of pure vitamin B₁ hydrochloride (*i.e.*, 333 I.U. ≡ 1 mg. or 333,333 I.U. ≡ 1 gram). The Permanent Commission on Biological Standardization of the Health Organization of the League of Nations adopted this recommendation. The International Standard now consists of pure crystalline vitamin B₁ hydrochloride, which is kept at the National Institute for Medical Research, Hampstead. The mean value of nine laboratories using the rat growth method was 817.2 I.U. per milligram of vitamin B₁ hydrochloride with a molecule of water of crystallization [28].

* 1,000,000 micrograms (µg) = 1,000 milligrams = 1 gram.

This is the value, now approximated to 820 I.U., accepted by the British Pharmacopœa.

The following table gives the approximate equivalents of the various units that may be met with in the older literature :—

1 mg. pure vitamin B ₁	≡ 333 International units.
	≡ 166 Smith curative units.
	≡ 666 Chase-Sherman units.
	≡ 333 Roscoe units.
1 International unit	≡ 0.5 Smith curative unit.
	≡ 2 Chase-Sherman units.
	≡ 1 Roscoe unit.
	≡ 20 milligram-equivalents (Cowgill).
	≡ 3.333 μg vitamin B ₁ .
1 Smith unit	≡ 2 International units.
	≡ 4 Chase-Sherman units.
	≡ 6.666 μg vitamin B ₁ .
1 milligram-equivalent (Cowgill)	≡ 0.05 International unit.

DISTRIBUTION OF VITAMIN B₁ IN FOODS

Vitamin B₁ is widely distributed in raw untreated foodstuffs, the richest sources being whole cereals, yeast, pork and pulses. Actually, however, there are few common foods that are potent sources, and in planning diets one must rely on several foods to obtain the optimum requirements of the vitamin. In preparing foods for the table considerable losses in vitamin content may occur through destruction by heat; by solution of the water soluble vitamin in the cooking water which may be, and usually is, thrown away; and by the rejection of bones, vegetable peelings and fruit cores. There are also considerable variations in the time taken to cook food and the method of cooking.

Vegetables, including potatoes, constitute an important group of foodstuffs as sources of vitamin B₁. Excluding legumes, they are not rich sources, but they are consumed regularly and by the bulk of the population. In both wheat and rice grain the vitamin is concentrated in the germ and outer or bran layer, and to a less extent in the aleurone layer. Osborne and Mendel [57] found that the endosperm adherent to the germ was also rich in the vitamin. The cereals rank first as the most important source of vitamin B₁ in human diets. On account of their cheapness and high calorie value they are consumed by most people. Refined cereals and flours, however, suffer considerable loss in their vitamin B₁ content because the germ and bran are largely removed in the milling. There is an increase in the vitamin B₁ content of cereals on germination, *e.g.*, from 7 μg to 9 μg per gram in the case of wheat.

White flour contains relatively little vitamin B₁ in comparison with wholemeal flour. It has been argued that the addition of yeast to white flour in the preparation of bread remedies this low vitamin B₁ content and renders white bread fit for consumption by the masses. The amount of

yeast added to bread is so negligible (1 of yeast to 100 of flour) that it can make very little difference to the final vitamin B₁ content. White bread only contains 45 μ g to 90 μ g of vitamin B₁ per 100 grams, *i.e.*, about a fifth to a tenth of that originally present in the whole wheat. "Germ bread" (*e.g.*, Hovis), a proprietary foodstuff prepared from three parts of white flour and one of wheat germ, is approximately equal to wholemeal bread in its vitamin B₁ content. Stoneground flour contains the whole germ and endosperm and the inner layers of the pericarp, which give a cream-coloured flour and not a brown one. "Peeled wheat" is prepared by a flotation process which removes only the thin epidermis and leaves ninety-eight per cent. of the wheat berry. It has a high content of vitamin B₁ and other members of the vitamin B complex [17].

In 1940 the Ministry of Food arranged for the fortification of white flour with vitamin B₁ to bring the vitamin B₁ content up to 0.088 mg. (28 I.U.) per 100 grams of bread. Owing to the lack of shipping space the Ministry forbade the milling of white flour after March 28rd, 1942, and the sale of white bread after April 6th, 1942, and replaced white flour, which only contains seventy-three per cent. of the wheat berry, by flour of eighty-five per cent. extraction. This flour, which is not fortified with vitamin B₁, is known as National flour in England and contains an average of 300 μ g to 400 μ g of vitamin B₁ per 100 grams. The loaf made from this contains 240 μ g to 255 μ g per 100 grams. On October 1st, 1944, a flour of 82.5 per cent. extraction was introduced by the Ministry of Food. In the early part of 1945 the extraction was lowered to 80 per cent. to satisfy the public. In the United States the millers may enrich white flour if they desire. Such flour must be labelled "enriched" and satisfy the following specification: Each pound must contain not less than 1.66 mg. and not more than 2.5 mg. of vitamin B₁ (or 0.47 to 0.7 mg. per 280 lb. sack); not less than 1.2 mg. and not more than 1.8 mg. of riboflavin; not less than 6 mg. and not more than 9 mg. of nicotinic acid or its amide; and not less than 6 mg. and not more than 24 mg. of iron. These additions are obligatory but the following are optional. Vitamin D may be added between the limits of 250 and 1,000 U.S.P. (International) units, and calcium between 500 and 2,000 mg. per pound of flour.

Legumes and nuts are important sources of vitamin B₁. Eggs contain a fair amount, although the content depends on the diet of the bird. Meat, apart from pork, is not a rich source. Liver extracts, however, may contain considerable vitamin B₁. Elvehjem [194] and his collaborators have shown that meat may serve as an adequate source of vitamin B₁, and may supply up to one-third of the daily requirements when it forms only ten per cent. of the diet. This is in accord with the observations of Stefansson [195], who noted that northern people consuming large quantities of meat never suffer from B₁-avitaminosis.

Yeast is an exceptionally potent source of vitamin B₁, and is frequently used as a dietary supplement when large quantities of the vitamin are required. Marmite is a commercial yeast extract. Brewer's yeast is more active than baker's yeast.

Milk is a poor source of vitamin B₁, an average sample of raw cows' milk containing about 40 μ g to 50 μ g per 100 c.c. The diet of the cow seems

to be without influence as the excretion of vitamin B₁ is under some kind of "physiological control," and it is certain that cattle can synthesize the vitamin in their intestines. It was shown by Japanese workers [58] that *B. coli* can synthesize vitamin B₁ in suitable media, and it is now considered that this occurs in the intestine of some animals, including cattle, under suitable conditions. Pasteurization of milk results in a loss of ten to twenty per cent. of the vitamin B₁ content, and commercial sterilization destroys some twenty-six to forty-five per cent. [59]. If the milk is concentrated by evaporation or drying the loss is greater still, *e.g.*, thirty to fifty per cent., although in the presence of sugar, as in sweetened condensed milk, the loss is only ten per cent. [63]. Human milk is also poor in vitamin B₁. In fact it is possible that many nursing mothers are unable to supply sufficient of this vitamin to their babies [60]. The vitamin B₁ content of human milk gradually increases until the twelfth week and then remains constant [759].

The mammary gland appears to offer a resistance to the transfer of vitamin B₁ from the blood to milk. Thus Morgan and Haynes [203] found that the vitamin B₁ content of human milk was only raised by vitamin supplements when the original level was very low. The administration of vitamin B₁ to nursing mothers had no effect on the vitamin B₁ content of the milk if this was originally normal. On an intake of 1.5 mg. daily a nursing mother secretes milk containing 20 μ g or 7 I.U. per 100 c.c. [204]. Colostrum contains practically no vitamin B₁.

Cowgill [61] comments on the high vitamin B₁ content of sugar-cane molasses (2.1 mg. per 100 grams), which is as great as that of some yeasts. According to him the negroes of the Southern American States keep free from beriberi because their diet contains molasses, pork and corn wholemeal, all of which are rich in vitamin B₁.

Effect of Cooking, Canning, Freezing and Drying on vitamin B₁. Many of the vitamin assays are based on the raw foodstuff, but by the time the latter is prepared for the table it may have suffered some loss in its vitamin B₁ potency. The chemical principles involved in the destruction of the vitamin by cooking have been previously discussed (p. 151). Generally speaking, the degree of inactivation is directly proportional to the temperature, duration of heating, and alkalinity of the medium. For all practical purposes there is little inactivation in acid or neutral solutions heated to 100° C. or just over for one hour. Higher temperatures and increased alkalinity (increased pH) hasten the rate of destruction; thus fifteen per cent. of the vitamin B in yeast is destroyed when it is heated for two hours at 180° C.

The destruction of vitamin B₁ when foods are cooked in the ordinary way is not appreciable, provided that the addition of soda to vegetables be rigorously excluded. In the case of peas moderate amounts of soda do not seem to cause excessive destruction of vitamin B₁ [64]. The average loss in the boiling of vegetables is about twenty to thirty per cent. [205]. This is reduced to ten per cent. by cooking with as little water as possible and retaining much of the steam. Much vitamin B₁ is dissolved out in the cooking water (twenty to thirty-five per cent.) and may be lost unless incorporated in soups and stews. Root vegetables, presenting less surface,

suffer less. Potatoes suffer less loss if cooked in their skins, retaining about ninety per cent. of their vitamin B₁. A considerable quantity resists aqueous extraction in starchy vegetables owing to adsorption on the starch grains. The loss is high if potatoes are steamed and the cooking liquid rejected, as a greater part of the vitamin B₁ is washed out [27]. The more succulent vegetables ("greens") do not retain so much of their vitamin B₁ on boiling. The widespread practice of using baking soda in cooking is to be condemned since it destroys much of the vitamin B₁. In cooking rice all the water should be evaporated. Running water over the cooked rice to separate the grains washes out much vitamin B₁. As much as sixty per cent. of the vitamin B₁ may be lost in this way [26]. Pressure cooking, although it may be rapid, causes considerable destruction of vitamin B₁.

In the baking of bread the loss of vitamin B₁ is variously reported from eight to thirty-three per cent. [65, 66]; it increases with the time of baking, *e.g.*, seventeen per cent. at twenty minutes and thirty-three per cent. at forty minutes. An average figure under normal conditions of large scale baking is twenty per cent. Dawson and Martin [65] give the following figures for the percentage losses of vitamin B₁ in the baking of various breads: white bread, eight to twenty-two; National loaf, twenty-seven; wholemeal ("brown" bread), thirty-five; germ bread, nineteen. A loss of thirty per cent. in the vitamin B₁ content occurs when baking soda is used [646]. When bread is toasted considerable destruction occurs in the outer part of the bread, although the total loss is not great. Toasting wholemeal bread for seventy seconds causes a drop in the vitamin B₁ content from 3.86 μ g per gram to 2.29 μ g per gram [49]. The cooking of oatmeal results in very little loss—five to ten per cent. in half an hour [19].

The loss of vitamin B₁ that occurs in foods cooked in restaurants and cafeterias has been studied by a number of investigators, particularly in America. Losses varying from sixteen to seventy-five per cent. have been reported [48]. Nagel and Harris [48] compute that twenty per cent. of the vitamin B₁ is lost in the cooking, twenty-five per cent. in the cooking water, and twenty-five per cent. between the time of cooking and serving. In the cooking of meat it has been estimated that there is about thirty per cent. loss on roasting and boiling and fifty per cent. on braising [202]; drying, being a quick process results in less destruction, *e.g.*, ten to forty-five per cent [194].

In the process of canning there should be little loss of vitamin B₁ due to the processing *per se* [67]. Any loss in potency usually occurs during the preparation of the food before it is put into the cans. As a rule fruits are processed at 100° C. or a little more, vegetables at 112°–115° C., and meat and milk at 117°–120° C. Provided the medium is not alkaline there should be no appreciable destruction of the vitamin B₁ if the heating is limited to half an hour. It is understood, of course, that the juices as well as the solid food are consumed, as much of the vitamin (thirty per cent.) is in the former. Baker and Wright [68] have shown that cooked and canned food-stuffs can be good sources of vitamin B₁. Arnold and Elvehjem [202] report a loss of up to twenty per cent. in tinned meat, and Rice and Robinson [906] thirty per cent. in canned ham, and Clifcorn and Heberlein

[898] give losses of thirty-four to seventy-four per cent. in canned vegetables. The loss in canning is increased on storage, and may be as much as forty-five per cent. after a year [23].

Foods preserved by freezing suffer little or no loss in their vitamin B₁ content [67, 69, 70]. The losses that do occur are due to the preliminary blanching or cooking rather than to refrigeration. Thus in the processing losses of five to twenty-five per cent. may occur [22]. It should be remembered that in the thawing process cell membranes are ruptured, and that when frozen foods are cooked vitamin B₁ and the other water soluble vitamins are more readily extracted by the cooking water than from the fresh product.

Dehydrated vegetables and meat have recently been introduced as a wartime measure to save space in transport. The loss of vitamin B₁ in dehydration is never more than about fifteen per cent. in vegetables, and most of this occurs during the blanching and not from the actual drying [20, 21]. In dehydrated meat the loss is from twenty-five to thirty-five per cent. [906], although figures as high as fifty per cent. are quoted [942].

In the preservation of vegetables by salting and brine preservation appreciable amounts of vitamin B₁ are retained [918].

Vitamin B₁ Content of Foods. The vitamin B₁ content of some of the more important foodstuffs in the raw and cooked states is given in the following table taken largely from the figures of Fixsen and Roscoe [71, 72], Booher and Hartzler [73], and Munsell [16] :—

Vitamin B₁ Content of Foods

Foodstuff.	Description.	Vitamin B ₁ Content.	
		Micrograms * per 100 grams.	International Units per 100 grams (= approx. 3½ ounces).
VEGETABLE PRODUCTS			
<i>Breads</i>			
Maize		240	80
Rye	Whole grain	240-500	80-167
	Germ	225	75
Wheaten bread .	Whole grain (wholemeal) .	225-450	75-150
	White bread (78% ex- traction) .	45-90	15-30
	„ „ baking powder .	81-63	10-21
	„ „ with malt . . .	81-105	27-85
	“ With germ ” brown bread .	240-270	80-90
	“ Without germ ” „ . .	195-240	65-80
	Bran bread	150	50
	“ Germ ” bread	240-510	80-170
	-Milk bread	90	30
	Ministry of Food loaf (85%) extraction (1942-44)	240-255	80-85
<i>Cereals and Cereal Products</i>			
Barley	Whole grain	500	167
	Germ	4,200	1,400

* Microgram (μg) is 1/1000th milligram or 0.000001 gram. One international unit = 3μg.

Foodstuff.	Description.	Vitamin B ₁ Content.	
		Micrograms * per 100 grams.	International Units per 100 grams (= approx. 3½ ounces).
<i>Cereals and Cereal Products—continued</i>			
Buckwheat . . .		450	150
Maize . . .	Whole grain . . .	185-180	45-60
	Germ . . .	1880	400
Oatmeal . . .	Whole grain . . .	540	180
	"Breakfast" . . .	420-810	140-270
Rice . . .	Whole . . .	60-270	20-90
	Polished . . .	30	10
	Bran . . .	1680-2280	560-760
Rye . . .	Whole . . .	300-500	100-167
	Germ . . .	2250	750
Sorghum . . .	Black . . .	270	90
(Kaffir corn)	White . . .	240	80
	Seed husk . . .	735	245
Wheat . . .	Whole grain . . .	540-1022	180-340
	White flour . . .	60-90	20-30
	Peeled wheat flour . . .	580	193
	"Germ" flour . . .	360-390	120-130
	Bran . . .	1080	360
	Germ (commercial) . . .	1,800-3,750	600-1,250
	Middlings . . .	1,350-1,675	450-525
	Stone ground . . .	480	160
	" " "white" . . .	270-330	90-110
	85% extraction (National Wheatmeal 1942-44) . . .	300-400	100-135
<i>Prepared Proprietary Cereal Foods</i>			
All bran . . .	—	370-520	123-173
Bemax . . .	—	2,625	875
Cerevim . . .	Vitamin concentrate added	2,100	700
Corn flakes . . .	Kellogg's . . .	390-450	130-150
	(Vitamin concentrate added)		
" " . . .	Post's . . .	280-400	98-133
	(Vitamin concentrate added)		
Cream of rice . . .	—	160	53
Cream of wheat . . .	Vitamin concentrate added	410-680	137-227
Force . . .	—	40	13
Grape nuts . . .	Post's . . .	810	270
	(Vitamin concentrate added)		
Oats . . .	Quaker . . .	580-700	193-233
Rice Krispies . . .	Kellogg's . . .	450	150
Shredded wheat . . .	Kellogg's . . .	190-230	63-77
Soya wheat . . .	—	710	233
<i>Fruits</i>			
Apple . . .	Raw . . .	30-120	10-40
Avocado	90	30
Banana	50-160	17-53
Blackberry	30	10
Black currant	30	10
Cherry	45	15
Gooseberry	25	8
Date	90	30
Fig . . .	Fresh . . .	75	25
	Dried . . .	45-150	15-50
Grape	50	17
Grapefruit . . .	Juice . . .	45	15

* Microgram (μg) is 1/1000th milligram or 0.000001 gram. One international unit = 3 μg .

Foodstuff.	Description.	Vitamin B ₁ Content.	
		Micrograms * per 100 grams.	International Units per 100 grams (= approx. 3½ ounces).
<i>Fruits—continued</i>			
Guava	45	15
Lime juice	30	10
Melon	30-60	10-20
Orange	Juice	70-92	23-31
Peach	Fresh	40	13
	Canned	40	13
Pear	50	17
Pineapple	50	17
Prune	Dried	125	42
Raisin	Dried	33-77	100-230
Raspberry	30	10
Strawberry	25	8-3
Tangerine	70	23
<i>Nuts</i>			
Almond	240	80
Brazil	500	167
Chestnut	270	90
Coconut	Dried	trace	trace
Coconut	Fresh	30-60	10-20
Hazel	400-600	133-200
Peanut	300-960	100-320
"	Roasted	200	67
Pecan	500	167
Walnut	450	150
<i>Vegetables</i>			
Asparagus	180	60
Bean, butter	Dried	480	160
" haricot	"	156-400	52-120
" "	Cooked	120-180	40-60
" green	Canned	33	11
" string	198-450	66-150
" runner	75-225	25-75
Beetroot	Boiled	30	10
Broccoli	111	37
Cabbage	30	10
Carrot	Raw	60	20
	Canned	33	11
Cauliflower	Raw	150-190	50-63
	Cooked	90	30
Celery	trace	trace
Cress	150	50
Cucumber	30	10
Endive	50-75	16-7-25
Kale	150	50
Kohlrabi	50	17
Leek	80	27
Lentil	120-630	40-210
Lettuce	75	25
Mango	60	20
Marrow	30-60	10-20
Mushroom	60	20
Okra	120	40
Onion	Stewed	30	10
Parsnip	80	2
Pea	Fresh	400-800	133-267
	" cooked	200	67

* Microgram (μg) is 1/1000th milligram or 0.000001 gram. One international unit = $3\mu\text{g}$.

Foodstuff.	Description.	Vitamin B ₁ Content.	
		Micrograms * per 100 grams.	International Units per 100 grams (= approx. $3\frac{1}{2}$ ounces).
<i>Vegetables—continued</i>			
	Dry	550-590	183-197
	" cooked	45-185	15-45
	Canned	360	120
Potato	Raw	90-180	30-60
	Peeled and boiled	90	30
Pumpkin	"	45	15
Radish	"	60	20
Rhubarb	"	15	5
Soya bean	"	520-1200	175-400
Spinach	Raw	56-100	19-33
Sprouts	"	180	60
Tomato	Pulp raw	70	23
Turnip	Raw	26-100	9-33
Watercress	"	100	33
Mushrooms	"	110	37
DAIRY PRODUCTS			
Cheese, Cheddar	"	24	8
Milk	Cow's fresh, whole	41-48	14-16
	" pasteurised	30	10
	" " boiled	24	8
	" whole, dried	210-270,	70-90
	Human milk	15-20	5-7
	Buttermilk	30	10
	Condensed, sweetened	120-186	40-60
	Evaporated	18-27	6-9
Eggs	Hen's, yolk, raw	300-420	100-140
	" " boiled, 5 mins.	300-420	100-140
	" white	trace	trace
	" dried	420	140
	" whole	150	50
	Duck's, yolk, raw	300	100
FISH			
Cod	Muscle	60	20
"	Liver	270	90
"	Roe	900-1,800	300-600
Clam	"	25	8-8
Crab	"	90-120	30-40
Dogfish	Liver	210-540	70-180
Haddock	"	60	20
"	Liver	750-2,190	250-730
Halibut	Fried	90	30
Herring	Whole	24-60	8-20
"	Roe (soft)	30	10
"	Smoked	111	37
Mackerel	Muscle	90	30
"	Roe	600	200
Oyster	"	300	100
Prawn	Boiled	< 90	< 30
Salmon	"	180-240	48-80
" Canned	"	24-45	8-15
Sardines	Tinned	90	30
Whiting	Muscle	90	30
Average lean fish	"	90-180	30-60

* Microgram (μg) is 1/1000th milligram or 0.000001 gram. One international unit = $3\mu\text{g}$.

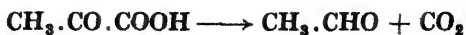
Foodstuff.	Description.	Vitamin B ₁ Content	
		Micrograms * per 100 grams.	International Units per 100 grams (= approx. 3½ ounces).
MEAT			
Beef	Average lean, raw	90-800	30-100
"	Average lean, cooked	72-240	24-80
"	Dried	180-600	60-200
" brain	"	168	56
" heart	"	675	225
" kidney	"	120-150	40-50
" liver	Raw	450	150
"	Cooked	198-450	66-150
" pancreas	"	318	106
Veal	"	105	35
Chicken	Muscle, roast	90-150	30-50
Lamb	Lean, raw	200-280	67-98
"	" roast	140	47
" liver	"	300	100
" heart	"	300	100
" kidney	"	120	40
" tongue	Braised	60	20
Pork	Lean, raw	960-1,200	320-400
"	" cooked	900	300
"	Salted	360	120
" ham	Boiled, smoked	660-1,070	220-357
" kidney	"	1020	340
" liver	"	270-468	90-156
" bacon	"	840-1,440	280-480
" brains	"	180	60
" lard	"	150	50
Rabbit	Stewed	60-120	20-40
MISCELLANEOUS			
Beer	"	9	3
" Bemax "	"	2,625	875
Chocolate	"	640	213
Coffee bean	Ground	420	140
Honey	"	5.5	2
Macaroni	"	45	15
" Malt "	Extract	300-475	100-165
" Marmite "	"	2,400-3,000	800-1,000
Molasses	Cane	2,100	700
"	Beet	360	120
Spaghetti	"	45	15
Tapioca	"	9	3
Yeast	Baker's	980-6,000	310-2,000
"	Brewer's	7,500-24,000	2,500-8,000
"	Extract	2,000-20,000	666-6,666
"	" D.C.L." (Distillers Co. Ltd.).	90,000	30,000
"	<i>Torulopsis utilis</i>	2,000	666

* Microgram (μg) is 1/1000th milligram or 0.00001 gram. One international unit = $3\mu\text{g}$.

THE PHYSIOLOGY OF VITAMIN B₁

Vitamin B₁ and Carbohydrate Metabolism. Vitamin B₁ has been shown to catalyse the decarboxylation and carboxylation of pyruvic acid, an intermediary degradation product of carbohydrate metabolism, both

in alcoholic fermentation and in tissue metabolism. In anaerobic fermentation by yeast pyruvic acid is decarboxylated to acetaldehyde and carbon dioxide :—

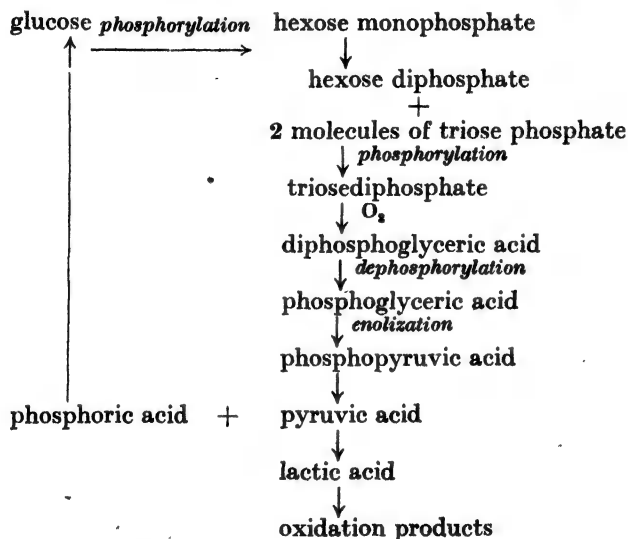


Yeast contains an enzyme, cocarboxylase or phosphothiamin, catalysing this reaction. In 1937 Lohman and Schuster [90] isolated pure cocarboxylase from bottom yeast and found that it was vitamin B₁ pyrophosphate. The same investigators have synthesized cocarboxylase chemically and others have obtained it by enzymic synthesis [91, 97]. The phosphorylation of vitamin B₁ to cocarboxylase is enzymic and is probably accomplished through the agency of adenylic acid and adenosine-triphosphate [96, 97], and not through the adrenal cortex hormone as formerly suggested [95] :

Adenosine triphosphate + vitamin B₁ → adenylic acid + cocarboxylase.

Phosphorylation is catalysed by the enzyme phosphorylase, which has been isolated in a chemically pure form [814].

Glucose is not directly oxidized in the body. The successive stages in its anaerobic oxidation (glycolysis) are probably as follows :



It can be seen that there is a progressive phosphorylation and hydrogen transfer starting with sugar through an intermediate series of compounds until pyruvic acid is formed.

The various steps in the breakdown of glucose are catalysed by enzymes which are activated by coenzymes. The enzymes are synthesized in the body, but the coenzymes, or at any rate their precursors, can only be made from dietary sources. Among the coenzymes essential for the degradation of glucose are :—

(1) Adenosine triphosphate, derived from adenylic acid (p. 108), which is a phosphate donor and acceptor.

(2) Diphosphopyridine nucleotide (codehydrogenase I) and triphosphopyridine nucleotide (codehydrogenase II), of which nicotinic acid is the precursor (p. 381). These coenzymes are hydrogen transporters.

(8) Magnesium.

The oxidation and decarboxylation of pyruvic acid requires the enzyme-coenzyme system carboxylase (protein-vitamin B₁ pyrophosphate-magnesium) and cocarboxylase, or vitamin B₁ pyrophosphate. There are other factors essential for the oxidation of pyruvic acid, including flavo-protein (p. 314), codehydrogenases I and II, adenosine triphosphate and the cytochrome system.

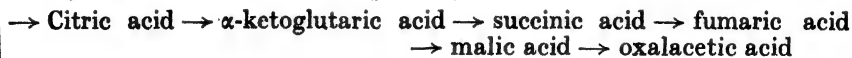
Pyruvic acid is not directly oxidized to lactic acid, but undergoes a series of complex changes. It was formerly suggested [92, 98, 146] that the change is a dismutation, and not a decarboxylation into lactic and acetic acids :



The evidence for this was based mainly on studies with bacteria. The available evidence at the moment is that pyruvic acid is carboxylated to oxalacetic acid [74], which in turn is utilized in two cycles, the citric acid and succinic acid.

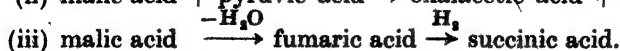
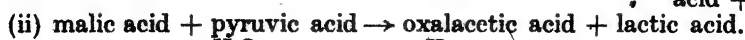
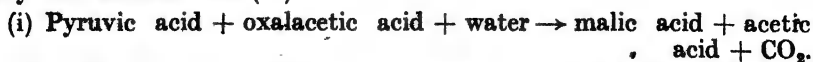


The enzyme catalysing this reaction, carboxylase, has recently been obtained in a highly purified form [75] and has been found to be a protein-vitamin B₁ pyrophosphate-magnesium complex. According to the citric acid cycle [80] pyruvic acid condenses with oxalacetic acid to form citric acid, which in turn is broken down by a series of enzymatic reactions to oxalacetic acid. The net result is the degradation of pyruvic acid, with oxalacetic acid as an intermediary. The rate of pyruvic acid oxidation is controlled by the presence of vitamin B₁. *In vitro* the synthesis of citric acid from pyruvic acid in the presence of kidney tissue is accelerated by vitamin B₁, and *in vivo* vitamin B₁ deficiency in rats results in a decreased urinary excretion of citric acid [14, 89].



Pyruvic acid.

In the succinic acid cycle, (i) oxalacetic acid is reduced to malic acid at the expense of the oxidation of pyruvic acid [80]. The malic acid may either form oxalacetic acid and lactic acid by reacting with another molecule of pyruvic acid, or (ii) it may be converted into fumaric acid and finally into succinic acid (iii).



Some of these reactions have been shown to occur in the metabolism of certain bacteria and of animal tissues such as kidney, brain and liver.

Vitamin B₁ in the form of cocarboxylase is essential for the metabolism of pyruvic acid. The essential rôle played by vitamin B₁ in carbohydrate metabolism was first demonstrated by Peters and his associates at Oxford [78, 79]. They showed an increased formation of lactic and pyruvic acids in the brain tissue of pigeons suffering from vitamin B₁ deficiency, and a diminished oxygen consumption in this tissue respiring in presence of glucose and Ringer-phosphate solution. The addition of vitamin B₁ produced a considerable increase in the oxygen uptake. This effect, known as the *catorulin effect*, is correlated with the degree of vitamin B₁ deficiency and is catalytic. The work of Peters was confirmed in America by Sherman and Elvehjem [76]. The final proof that the active form of vitamin B₁ in pyruvic acid oxidation is cocarboxylase was supplied by Banga, Ochoa and Peters [143, 144]. They showed that cocarboxylase is far more potent than vitamin B₁ in producing the catorulin effect in the brain tissue of polynuritic pigeons. It remained to be explained why vitamin B₁ itself was active in the earlier studies by Peters; the activity was subsequent to its phosphorylation to cocarboxylase. There is now little evidence in support of the view that cocarboxylase acts as a hydrogen carrier in biological systems, through alternate oxidation and reduction of the quaternary thiazole nitrogen atom in vitamin B₁, although it may act in oxidation-reduction systems through its disulphide [8].

Vitamin B₁ becomes phosphorylated to cocarboxylase when added to many animal tissues [91]. According to Goodhart and Sinclair [94] the white blood cells originating in the bone marrow convert vitamin B₁ into cocarboxylase, which is then probably combined with a protein. The circulating form of vitamin B₁ is the free substance or its monophosphate, not cocarboxylase, which is probably formed mainly in the liver. At any rate there is a massive synthesis of it in the liver after the injection of vitamin B₁. Cocarboxylase in the liver is hydrolysed when necessary to replenish the blood vitamin B₁. The kidney also phosphorylates vitamin B₁, the process being probably essential for its reabsorption in the kidney tubules. It is interesting to note that the heart, which is a tissue known to be affected in avitaminotic conditions (*e.g.*, beriberi) shows a diminution of cocarboxylase corresponding with the presence of vitamin B₁ deficiency symptoms. According to Peters [56] vitamin B₁ is present in plants mainly in the free state, and in animal tissues as cocarboxylase.

Since the liver and kidney possess the power of phosphorylating vitamin B₁ to cocarboxylase, it would be expected that some disturbance of phosphorylation might occur in hepatic and renal disease. This has been demonstrated by Williams and Bissell [914]. They found that the injection of 15 mg. of vitamin B₁ intravenously caused a rapid increase in cocarboxylase and free vitamin B₁ in the blood of normal subjects. The vitamin B₁ level rapidly returned to normal, but the cocarboxylase remained elevated for an hour or so. In patients with advanced hepatic cirrhosis there was an immediate rise in the free vitamin B₁ in the blood, but the increase in cocarboxylase was considerably less than in normal subjects. In cases of nephritis the changes were intermediate between those in the normal subjects and those with hepatic disease.

Davis and Bauer [936] have also shown that there is some degree of

vitamin B₁ deficiency in patients with hepatic disease, as shown by elevated blood pyruvic acid levels (p. 236). They did not, however, find a raised blood pyruvic acid in patients with renal disease.

Further relationships between cocarboxylase and carbohydrate metabolism remain to be discussed. Through its effect on the oxidation of pyruvic acid it may influence the various phases of carbohydrate metabolism, since the oxidation of pyruvic acid causes the storage of a considerable amount of energy as adenosine triphosphate [4, 77]. It may be indirectly concerned in the synthesis of glycogen from glucose [677] and the conversion of fructose to glucose, reactions in which phosphorylation of the sugar are essential. Cocarboxylase would also appear to be essential for the synthesis of carbohydrate from lactic and pyruvic acids, probably by a reversal of the glycolytic process described on p. 163. It has been shown that the synthesis of carbohydrate from pyruvic acid is decreased in kidney slices of vitamin B₁ deficient rats and restored to normal by the addition of vitamin B₁ [173, 174].

Many investigations have been carried out to see if increased quantities of pyruvic acid and other intermediate products of carbohydrate metabolism can be detected in the blood of animals and human beings suffering from vitamin B₁ deficiency. The presence of pyruvic acid in the blood can be demonstrated by an increase in the bisulphite binding power (B.B.S.) of the blood. Pyruvic acid contains a ketonic group (CO) and therefore combines with sodium bisulphite. The test, not being specific for pyruvic acid, is given by other substances containing an aldehyde or ketone group, *e.g.*, methyl glyoxal, which is also an intermediate product of carbohydrate metabolism. Pyruvic acid has been found in abnormal amounts in the blood of animals suffering from vitamin B₁ deficiency and the level returns to normal after treatment of the latter [82, 83]. This has also been demonstrated in human beings suffering from beriberi and related deficiency diseases [84, 85]. Light muscular exercises in patients suffering from vitamin B₁ deficiency causes the level of blood pyruvate to rise still further [86, 88]. The blood pyruvate rises measurably after exercise in untrained normal persons, but the blood lactate does not [102], in contrast to patients with beriberi and suffering from vitamin B₁ deficiency, who show both a raised blood lactate and pyruvate level. Chesler, Homburger and Himwich [919] noted a high post-absorptive blood sugar, a rise in the blood lactic and pyruvic acids, and a lowering of the lactic acid pyruvic acid ratio in vitamin B₁ deficiency. There is considerable difference of opinion as to whether the blood pyruvate level is of value for the biochemical detection of vitamin B₁ deficiency (p. 236).

Observations on the effect of vitamin B₁ on carbohydrate metabolism *in vivo* are of a conflicting nature. There are a number of complicating factors to be considered in this connection. One is that inanition accompanies vitamin B₁ deficiency and results obtained may be due, in part at any rate, to this. Another is that other factors of the vitamin B complex are involved; this must be considered particularly in clinical reports concerning patients with beriberi and vitamin B₁ deficiency, which is always associated with lack of the other B factors. Demole and Silberschmidt [98] state that the administration of 0.1 to 4 mg. of vitamin B₁ has no effect

on the blood sugar level of normal rabbits nor on the temporary hyperglycæmia produced by injecting glucose. Other workers state that the effect of vitamin B₁ on the fasting blood sugar is irregular and may produce either a rise or fall [99, 105, 106, 107, 668, 669]. The evidence for the effect of vitamin B₁ on insulin hypoglycæmia is also conflicting; both a rise and a fall of blood sugar have been reported in animals [99, 100, 101, 104]. In the normal human being and in the diabetic the general opinion is that vitamin B₁ has no effect on the blood sugar level [108, 109, 185, 670, 754]. Its effect on the blood sugar after the injection of insulin appears to be variable; a rise, a fall and no effect have been reported [100, 109, 110, 185, 555, 615, 754]. Studies have also been made on the storage of glycogen and blood sugar levels in vitamin B₁ deficiency. A number of workers have reported a rise of blood sugar and a failure to deposit liver glycogen in avitaminosis B₁ [106, 380]. There is evidently a species difference as the blood sugar falls and later rises in dogs suffering from avitaminosis B₁, whereas in pigeons hyperglycæmia predominates throughout [107]. Martin [108] believes that vitamin B₁ and riboflavin play a fundamental rôle in maintaining the blood sugar level. He administered insulin to depancreatized dogs on diets free from vitamin B₁ and riboflavin, but the animals still suffered from hyperglycæmia and glycosuria. The administration of vitamin B₁ produced no response. If given with riboflavin, however, hypoglycæmic shock resulted. Tonutti and Wallraff [106] state that glycogen is absent from the livers of B₁-avitaminotic rats. Injections of vitamin B₁ and glucose brought about an accumulation of liver glycogen.

In both animals and human beings living on a diet deficient in vitamin B₁ there is a decreased glucose tolerance [327, 330], and in animals at any rate a decrease in the rate of absorption of glucose from the intestine [780].

These results are so contradictory that they negate any specific relationship between vitamin B₁, blood sugar, and glycogen storage. The clinical aspect is discussed on p. 268.

The most striking lesions in animals and human beings suffering from a pure vitamin B₁ deficiency are in the nervous system, the cells of which utilize only carbohydrate for their energy [175]. It is supposed that lack of vitamin B₁ results in inefficient metabolism of carbohydrate throughout the tissues, including the nervous system. The oxidation of carbohydrate by nerve tissue is not only depressed in vitamin B₁ deficient subjects [105], but also by alcohol, anæsthetics and narcotics, which are stated to increase the vitamin B₁ requirements of the organism considerably [678].

It must not be supposed that vitamin B₁ functions specifically in nervous tissue only; it affects metabolism in general. The catatorulin effect has not only been demonstrated in brain but in other tissues such as kidney, heart and liver.

Vitamin B₁ and Fat Metabolism. In animals a diet poor in vitamin B₁ and rich in carbohydrate brings on the symptoms of vitamin B₁ deficiency more rapidly than a diet rich in fat [111, 141, 142]. Banerji and Yudkin [287] state that rats can thrive on a diet free from vitamin B₁ provided it contains no carbohydrate. They have shown that both protein and fat have a vitamin B₁-sparing action. The vitamin B₁-sparing action of protein

has been confirmed [351]. Animals suffering from vitamin B₁ deficiency will also eat fat in preference to carbohydrate if a choice of diet is offered them [225]. When fat is substituted isocalorically for carbohydrate in the diet of rats suffering from vitamin B₁ deficiency, there is a decrease in bisulphite-binding substances in the urine [580, 581]. The vitamin B₁ requirements of an animal are less on a diet rich in fat than on one containing much carbohydrate.

The rôle of vitamin B₁ in the metabolism of carbohydrate renders this vitamin B₁-sparing action of fat intelligible since the vitamin is needed for the oxidation of carbohydrates, but not for that of ingested fats. According to McHenry [114] the presence of choline is necessary for the vitamin B₁-sparing action of fat. Evans and his collaborators [112] have arranged fats according to their vitamin B₁-sparing action, the efficiency of the fat depending on the length of the fatty acid chain, maximum protection being reached with fatty acids containing eight carbon atoms. On the other hand fat synthesis from ingested carbohydrate appears to depend on the presence of vitamin B₁ [118] and other members of the vitamin B complex [478]. Banerji and Yudkin [287] do not consider that the results of vitamin B₁ deficiency are due to defective carbohydrate metabolism because they have observed the catatorulin effect, *i.e.*, increased oxygen uptake by tissues in the presence of pyruvate, in the absence of vitamin B₁. They explain the ill-effects of vitamin B₁ deficiency rather by the presence of a toxic substance produced during an incomplete or perverted carbohydrate metabolism than by the inability *per se* to carry out a metabolic process essential for normal physiological function. They have shown that the metabolic disability is present in any animal deprived of the vitamin and that symptoms of vitamin B₁ deficiency appear only when it receives carbohydrate in its diet. Their animals thrived on a diet free from vitamin B₁ and containing protein and fat; when they were given carbohydrate deficiency symptoms were produced. They consider that these symptoms are most likely due to toxic products of perverted metabolism, such as methyl glyoxal or adenylic acid.

In man the results are equivocal. Thus Wiedenbauer and Wieland [961] state that there is increased utilization of vitamin B₁ following the consumption of high carbohydrate diets and Wang and Yudkin [167] record a reduced excretion of vitamin B₁ in subjects receiving an increased intake of carbohydrate. On the other hand, Cahill [917] could find no difference in the urinary vitamin B₁ excretion during alternate periods of high fat and high carbohydrate intake. Reinhold, Nicholson and Elsom [918], writing in 1944, state that they were unable to find any evidence for the vitamin B₁-sparing action of fat in man; in fact the urinary excretion of vitamin B₁ was decreased in five out of six subjects when the fat ration was increased. Their observations confirm the view that the amount of carbohydrate in the diet is an important factor in determining the daily requirements of vitamin B₁.

These conflicting results are probably due to the difficulty of keeping human diets constant in one factor while varying another.

Vitamin B₁ and the Endocrine System. Thyroid. Most of the studies on the relationship between thyroid function and vitamin B₁ are experi-

mental, although a few clinical studies have been made. Experimental observations can be divided into: (a) the effect of vitamin B₁ deficiency on the thyroid gland, (b) the effect of the administration of vitamin B₁ on animals given desiccated thyroid or thyroxine or the thyrotropic hormone of the anterior pituitary gland. The literature on the effect of vitamin B₁ deficiency on the thyroid gland is conflicting. It is well reviewed by Drill [279]. After prolonged vitamin B₁ deficiency structural changes in the thyroid gland have been described, characterized by an increase in colloid [115] and by hyperplasia [289], followed by atrophy of the gland as the deficiency was prolonged. Other investigators [240] state that no changes in the thyroid gland can be detected in animals suffering from vitamin B₁ deficiency, and attribute the increased colloid formation to iodine deficiency. Similarly it is variously stated that injections of vitamin B₁ stimulate the thyroid and also have no effect on the gland [116, 482]. Hyperthyroidism increases the requirements of vitamin B₁ (p. 190), but the effect is not specific as hyperthyroidism results in increased metabolism, which is known to increase the requirements of a number of vitamins, including B₁ and C.

The loss in weight and anorexia produced in animals by feeding thyroid gland or thyroxine can be corrected by the administration of vitamin B₁ [117-120, 644]. Doses of 100 μ g of the latter can annul the effect of 0.2 mg. of thyroxine in the experimental animal. Experimental hyperthyroidism is accompanied by a fall in tissue cocarboxylase [120]. Drill and Sherwood [118] showed that the effect of vitamin B₁ in preventing the loss in weight of hyperthyroid dogs is due to the increased caloric intake which it produces. It also appears that not only vitamin B₁ but other members of the vitamin B complex are needed for the recovery of lost weight in hyperthyroid animals [278]. Liver function, including glycogen storage, is also depressed in hyperthyroid dogs, and this is intensified by removing the vitamin B complex from their diet. A diet rich in the vitamin B complex delays but does not prevent the onset of damaged liver function [295]. Hyperthyroidism decreases the amount of vitamin B₁ in various rat tissues. Summarizing the animal work it may be said that in hyperthyroid animals there is an increased demand for vitamin B₁, which if not supplied results in a depletion of the body stores of the vitamin, with resulting anorexia, loss of weight, decreased stores of glycogen and diminished hepatic function. These changes can be prevented by vitamin B₁ and the B complex.

A number of clinical observations on the subject have been made. It is generally agreed that there is an excessive urinary excretion of vitamin B₁ in thyrotoxic patients [296], and Frazier and Ravdin [121] as well as Means [297] have pointed out that such patients often show symptoms suggestive of vitamin B₁ deficiency. Williams and his co-workers [296] observed that the blood cocarboxylase was below normal and the blood pyruvate and lactate elevated in thirty-four of forty patients with thyrotoxicosis. The intravenous injection of glucose also resulted in a blood pyruvate level higher than normal. Williams and Kendall [298] state that patients given thyroid clinically tolerate it better if vitamin B₁ is given as well. From tests on normal volunteers they concluded that the thyroid

hormone is less effective in stimulating metabolism in a state of vitamin B₁ deficiency. Two normal subjects were given approximately 0.5 gm. of desiccated thyroid and placed on diets containing variable quantities of vitamin B₁. When the diet was adequate in vitamin B₁ the B.M.R. rose to + twenty-five per cent; it fell to between - eight and + eleven per cent when the vitamin was restricted, and rose to + twenty-five to + thirty per cent. when vitamin B₁ was again provided in adequate amounts.

Vitamin B₁ and Acetylcholine. Vitamin B₁ is an essential factor in the transmission of peripheral nerve impulses. It augments the activity of acetylcholine at nerve endings by inhibiting the formation of cholinesterase, an enzyme that hydrolyzes acetylcholine and inactivates it [122, 126]. In the isolated gut and heart vitamin B₁ augments the action of acetylcholine [128, 771]. This has been challenged by Erspamer [437] who claims that in concentrations of 1 in 10,000 vitamin B₁ antagonizes the action of acetylcholine. Glick, Antopol and others [124] showed that vitamin B₁ inhibits the action of cholinesterase in animal sera, but they point out that it is only effective in a concentration greater than that found in the tissues. They also observed that the blood cholinesterase is increased in pigeons with vitamin B₁ deficiency. Vitamin B₁ itself has no effect on smooth muscle, but acetylcholine, the acetyl ester, like acetylcholine, causes it to contract [147]. During nerve stimulation both vitamin B₁ and acetylcholine are formed [885], and it has been suggested that it is not the vitamin itself but the acetyl ester that is liberated. If acetylcholine, like acetylcholine, is a chemical intermediary in the propagation of the nerve impulse one would expect it to be rapidly removed from the site of action. Acetylcholine is rapidly hydrolyzed at the nerve ending by cholinesterase. Actually the enzymatic hydrolysis of acetylcholine by serum and brain extracts is very slow [124]. In the presence of pyruvic acid and potassium ions vitamin B₁ effects the synthesis of acetylcholine in brain tissue [128]. Most of the vitamin B₁ of nerve tissue is in the myelin sheath and this is the storage battery in which acetylcholine formation takes place.

By alkaline oxidation of nerve fibres vitamin B₁ can be demonstrated in the myelin sheath by the fluorescence of the resulting thiochrome [923]. In degenerated nerve a diminution in the vitamin B₁ content of the myelin sheath can be demonstrated in twenty-four hours. The acetylcholine content of nerve tissue decreases in the vitamin B₁ deficient animal [924].

Vitamin B₁ and Liver Function. Some workers have attempted to establish a relationship between vitamin B₁ and liver function. Thus it is stated that an injection of the vitamin will prevent a fall in liver glycogen after chloroform or ether anaesthesia [619, 648], and it has therefore been recommended for routine use before administering anaesthetics [619]. Mukherji [649] has also shown that vitamin B₁ protects rats against poisoning by guanidine, and his explanation is that it facilitates the detoxication of this compound in the liver. It is well known that pathological changes occur in the liver as a result of severe burns. Blotvogel and Tonutti [650] state that the administration of vitamin B₁ clinically in doses of 10 mg. minimises the toxæmia that commonly follows severe burns, and they claim that it acts by improving liver function. Only

seventeen patients were studied and there is no evidence that the work was controlled. No tests were made on the liver function of the patients.

The evidence that vitamin B₁ alone is an important factor in the preservation of liver function is slender. It is more likely that a depressed liver function interferes with the ability of the organism to store and utilize vitamin B₁ (p. 210). Certainly the excretion of vitamin B₁ is lowered in hepatic disease. There is evidence, however, that some factors of the vitamin B complex, particularly choline, can prevent fatty degeneration and cirrhosis of the liver in the experimental animal (p. 118). Liver function, including glycogen storage, is depressed in dogs treated with thyroid or thyroxine, and this effect is intensified if the vitamin B complex is removed from the diet, and diminished by feeding a diet rich in the vitamin B complex [295].

Œstrone is inactivated in the liver. If female rats are given a diet deficient in the vitamin B complex the amount inactivated is considerably reduced, although feeding with yeast prevents this [586]. The actual factor of the B complex responsible is unknown. This is interesting clinically as the occurrence of menorrhagia and metrorrhagia in women with cirrhosis of the liver has long been known and is referred to in Osler's "Modern Medicine" (1908). Menstrual disturbances have also been reported in other intoxications. Biskind [891] describes the successful treatment of menorrhagia, metrorrhagia and pre-menstrual tension associated with excess Œstrogen in twenty-two women by means of large doses of the B vitamins.

Vitamin B₁ and Hæmatopoiesis. There appears to be no direct relationship between vitamin B₁ and hæmatopoiesis. There is no disturbance of hæmatopoietic function in growing or adult animals on a restricted intake of vitamin B₁ [921].

Reproduction. Vitamin B₁ plays some part in the reproductive mechanism of the rat, since the fertility of this animal is seriously impaired if it is deprived of the vitamin [757]. Disturbances of lactation also result. Mice kept on diets deficient in vitamin B₁ are more susceptible to infection [758].

Sure [189] reports that exceptionally large doses of vitamin B₁ administered to rats produce partial sterility in the first generation and diminished lactation in the third generation. A similar effect was recorded by Perla [190], who found that feeding rats with excess vitamin B₁ resulted after one generation in interference with lactation, loss of maternal instinct, cannibalism and progressive loss of fertility. These symptoms were prevented by giving 2 mg. manganese chloride daily to the animals.

Anœstrus is also produced in rats suffering from vitamin B₁ deficiency [757], although it is claimed that this effect is due to concomitant inanition depressing the functions of the anterior pituitary gland [941]. It has been reported that the male and female sex hormones and vitamin D₁ delay the symptoms of vitamin B₁ deficiency [299]. Large doses of vitamin B₁ are stated to depress the activity of the anterior pituitary gland, as shown by diminished excretion of progesterone [800]. This observation suggests

the use of vitamin B₁ as an adjuvant to hormone therapy in the treatment of endocrine disturbances.

Vitamin B₁ and Phagocytic Function. Studies in vitamin deficiencies have shown that adequate amounts of most vitamins are essential for normal resistance to infection. Careful quantitative studies of variations in phagocytic power in different nutritional conditions have been made by Cottingham and Mills [989]. They find that the phagocytic activity of the peritoneal fluid in mice is diminished by eighty per cent. if the animals are suffering from a mild degree of vitamin B₁ deficiency. When this is severe phagocytosis cannot be demonstrated. Reduction in phagocytic power was also observed in mice suffering from a deficiency of other vitamins (pp. 108, 119, 820).

Vitamin B₁ and Mineral Metabolism. Perla and Sandberg [129, 180] believe that there is a metabolic interdependence of the vitamin with manganese, the latter acting as an oxidative catalyst in the utilization of vitamin B₁ in the tissues. Perla [190] also observed that vitamin B₁ deficiency caused an increased retention of manganese in rats, and that the toxic manifestations of an excess of vitamin B₁ in the diet could be prevented by small doses of manganese. The results of studies on iron and copper were not sufficiently constant to be reported. It has been observed that manganese in minute amounts stimulates the carboxylase system (p. 164) [768].

There appears to be some relationship between vitamin B₁ and zinc, which like manganese can replace magnesium in the carboxylase complex [764]. In beriberi the zinc content of the blood, nails and skin falls to half the normal values [765]. There would seem to be some correlation between the vitamin B₁ and zinc content of foodstuffs.

Relationship to Other Vitamins. The relationship of vitamin B₁ to other members of the vitamin B complex and to other vitamins has not been fully elucidated. The phosphorylation of vitamin B₁ is presumed to occur through the agency of adenylic acid (pp. 109, 168), and the oxidation of pyruvic acid is stated to require not only vitamin B₁ but pantothenic acid (p. 118) and biotin (p. 125). In animals suffering from vitamin B₁ deficiency there is a pronounced disturbance of riboflavin metabolism, the riboflavin content of the tissues falling considerably, mainly because of poor absorption [766]. There is, however, an increase in the riboflavin content of the liver in animals deficient in vitamin B₁ [921]. Clinically it has been shown by Sydenstricker [767] that in cases of nutritional deficiency resulting from lack of the whole vitamin B complex the administration of massive doses of vitamin B₁ may precipitate symptoms of a deficiency of one of the other members of the vitamin B complex, *e.g.*, nicotinic acid or riboflavin. The administration of large doses of vitamin B₁, *e.g.*, 10 mg. to 80 mg. over a period increases the urinary riboflavin excretion in man [768]. Vitamin A is stated to act antagonistically against vitamin B₁, since the symptoms of vitamin B₁ deficiency are intensified by giving vitamin A. In the rat, however, a deficiency of vitamin A results in an increase of blood pyruvic acid, which is a manifestation of vitamin B₁ deficiency, and which responds to the administration of vitamin B₁ [769]. A possible relationship exists between

vitamin C and vitamin B₁. It is said that the onset of scurvy on a minimal intake of vitamin C is delayed by small amounts of vitamin B₁, and that the antineuritic action of vitamin B₁ is increased by vitamin C [671]. The oral lesions of vitamin B₁ deficiency in dogs have been relieved by giving vitamin C [778].

Vitamin B₁ as a Growth Factor. Much has been written on vitamin B₁ as a specific growth factor. While it is essential for the growth of young animals and of many micro-organisms this is true of nearly all the vitamins of the B complex. It is doubtful if it has any effect on growth *per se*; it probably acts indirectly through its effect on normal metabolic processes. A young animal on a diet deficient in vitamin B₁ suffers from anorexia and loses weight. Feeding vitamin B₁ soon restores appetite and weight. A number of clinical investigations have been carried out to see how far the health and development of infants may be improved by increasing the vitamin B₁ content of their food. In much of the earlier work the vitamin B complex, in the form of wheat germ, rice polishings or brewer's yeast, and not pure vitamin B₁ was used. The general opinion was that infants receiving supplements of pure vitamin B₁ or of the vitamin B complex showed a greater rate of growth than controls not receiving vitamin supplements [178, 181, 184]. Macy and his collaborators [181] noted a greater growth rate in the infants given vitamin B₁ supplements, but the incidence of respiratory infections and progress in sitting, walking and talking were alike in the treated infants and controls. The observations on mental development were of considerable interest. At six months the vitamin B₁ group showed a distinct superiority over controls in such tests as visual pursuit, eye-hand co-ordination, and prehension. The differences were not so marked at nine and twelve months. Elias and Turner [179], however, failed to find an increased rate of growth in infants given supplements of wheat germ and yeast compared with controls. While the effects of supplements of vitamin B₁ on the increased rate of growth of infants in these studies are not doubted, it must be pointed out that it was not proved that the controls were receiving sufficient vitamin B₁. Their vitamin B₁ intake was not recorded. If an infant is receiving an adequate intake of vitamin B₁ for normal growth it is difficult to see what beneficial effect a further quantity can make.

Absorption, Storage and Excretion of Vitamin B₁. Vitamin B₁ is rapidly and completely absorbed from the small intestine, and probably from the large intestine, although evidence on this is conflicting. Thus Schroeder and Liebich [182] were unable to observe evidence of its absorption when administered through a cæcostomy and Linneweh and Müller [779] state it is not absorbed *per rectum*. The work of Najjar and Holt [780], however, suggests that it may be synthesized in and absorbed from the large bowel, and that it can be absorbed from a rectal enema. The vitamin may be secreted into the gastric juice [52]. Vitamin B₁, even if ingested in sufficient quantity, may escape absorption from the intestine in certain digestive disturbances such as vomiting, diarrhoea [133], alterations of the gastro-intestinal mucosa (ulcerative colitis) [134] and defective secretion due to atrophy, inflammation, and neoplastic disease. Operations designed to short circuit the intestines [135], internal and external fistulæ

and strictures will also lead to diminished absorption. In fact almost any pathological condition of the gastro-intestinal tract may cause it (p. 209). Diseases associated with diuresis may wash considerable quantities of vitamin B₁ from the tissues before it can be utilized. It is imperfectly absorbed in the patient with achlorhydria, as in this condition it is largely destroyed by the gastric juice [259]. For the complete absorption of vitamin B₁ by mouth it is necessary to have sufficient hydrochloric acid in the stomach. Even if the intake by mouth is adequate, the emptying time of the stomach plays a very important rôle in its absorption, because if food is hurried through the stomach into the alkaline duodenum the vitamin B₁ will be partially destroyed. The absorption and assimilation of vitamin B₁ in old persons is presumably not so efficient as much larger quantities of the vitamin are required for saturation [781].

Melnick and his co-workers [51] have shown that when an oral dose of vitamin B₁ is taken by a normal subject on an empty stomach, a much smaller fraction is excreted than when the same dose is ingested during or just after a meal. The difference may be due to the instability of the vitamin in a more alkaline gastro-intestinal tract when gastric secretion is at a minimum. *In vitro* studies show that it is stable in gastric juice, but is rapidly destroyed by bile, pancreatic juice and antacids.

Vitamin B₁ is probably not phosphorylated before absorption from the intestines [52]. Absorption occurs most likely by simple diffusion, because the amount absorbed is roughly proportional to the concentration in the intestine, and because the initial rapid absorption is succeeded by a much slower rate of absorption, presumably after diffusion equilibrium between the lumen of the gut and the intestinal mucosa has been established [58]. Hepatic disease may interfere with intestinal absorption as patients with this condition excrete less vitamin B₁ than normal [726].

The phosphorylation of vitamin B₁ occurs in all nucleated cells [94], although those of the liver and kidney are particularly active, since extensive phosphorylation occurs in these organs after the injection of vitamin B₁. The circulating form of vitamin B₁, *i.e.*, that presents in the plasma, cerebrospinal fluid and urine, is the free vitamin and not cocarboxylase. According to Goodhart and Sinclair [94] the white blood cells originating in the bone marrow convert vitamin B₁ into cocarboxylase, which is then probably combined with a protein. There is no evidence for the view put forward a few years ago that the adrenal cortex is essential for the phosphorylation of vitamin B₁. The available evidence indicates that vitamin B₁ reaching the blood stream from the intestine is taken up mainly by the liver and kidneys, where it is phosphorylated and stored as cocarboxylase [768]. All tissues, but mainly the liver and kidney, can dephosphorylate cocarboxylase to vitamin B₁ [91A] and supply the free vitamin to the blood; this is transported in the plasma [94] to other tissues, which rephosphorylate it more slowly [768] or else it is excreted in the urine. The bulk of vitamin B₁ in the urine is free, but a small amount of cocarboxylase is present as well. The total amount of vitamin B₁ in the human tissues averages 25 mg. The richest tissue is heart muscle (2 μ g to 3 μ g per gm.), followed by brain, kidney and liver (1 μ g per gm.) and skeletal muscle (0.5 μ g per gram) [189, 785].

Storage. The body is unable to store vitamin B₁ to any extent for any length of time, although the pig seems exceptional in this respect. Previous saturation of an animal with vitamin B₁ does not significantly prolong the time that it survives on a vitamin B₁ free diet (compare vitamins A, D, E). Losses of vitamin B₁ from the tissues when the animal is placed on a vitamin B₁ free diet do not occur equally rapidly from these organs. Thus the liver loses four-fifths of its vitamin in one week, and at the end of five weeks only a twentieth of it is left; the heart loses the vitamin far more slowly. The lungs, stomach, small intestine and spleen have lower vitamin B₁ contents, but in animals on deficient diets they show a more gradual loss than the tissues that are normally richer in the vitamin. Depletion occurs most rapidly in the muscles, slowest in the brain.

Vitamin B₁ occurs in skeletal muscle in two phases, extracellular and intracellular. The extracellular is probably freely diffusible and is in equilibrium with the vitamin B₁ of the plasma [908], the concentration in which is about 4 μ g per 100 c.c. The concentration of intracellular vitamin B₁ is considerably greater, being about 50 μ g per 100 gram. The vitamin B₁ present in muscle is thus almost entirely intracellular.

When the vitamin B₁ in the diet is gradually increased the concentration in the body eventually reaches a point of saturation which is not exceeded in spite of an increased intake [140]. When the body is saturated the greater amount of the vitamin is stored in the muscles. Actually a large part of the ingested vitamin B₁ is metabolized, possibly as much as ninety per cent. If more than the optimum needs are ingested the excess is wasted by destruction in the body or else it is excreted in the urine.

According to Sinclair [150] the concentration of vitamin B₁ in the blood of normally nourished persons varies from 5.5 μ g to 10.5 μ g per 100 c.c. of whole blood, with a mean of 7.4 ± 1.4 . This figure, which includes free vitamin and cocarboxylase, agrees with that of Widenbauer [168], but is lower than those obtained by other workers using a variety of methods [151, 152, 153]. Deutsch [723] gives a range of 9 μ g to 16 μ g per 100 c.c. Using the yeast fermentation method of estimation (p. 237), Goodhart [723] stated that the average vitamin B₁ content of blood is 5.39 μ g per 100 c.c., with a range of 3.1 μ g to 9.2 μ g. Most of the vitamin B₁ in blood is combined as cocarboxylase, but there is always 0.5 μ g per 100 c.c. in the free state. Goodhart and Sinclair [875] give the range of blood cocarboxylase as 4.5 to 12 μ g per 100 c.c., with a mean of $7.0 (\pm 2.1)$. Goodhart [417], using washed blood cells, found a mean value of 7.0 ± 1.58 . Schlutz and Knott [714] state that a healthy child should have a blood level of 10 μ g or more of cocarboxylase per 100 c.c. Wortis [601] puts it between 6.5 μ g and 8 μ g, and Benson and co-workers [783] give a range of 4.8 μ g to 12.8 μ g, with an average of $7.8 \pm 1.8\mu$ g. The leucocytes of the blood contain ten times as much vitamin B₁ as the erythrocytes, due no doubt to their more active metabolism [922].

In certain conditions such as alcoholic neuritis, nutritional neuritis, scurvy and malnutrition the vitamin B₁ level in the blood is considerably lowered. Widenbauer and his co-workers [168] found low values in pregnancy, lactation, hyperthyroidism and cancerous cachexia. Administration of vitamin B₁ to those with low blood values raised them to

normal, but rapid urinary excretion prevented the blood values from rising very high even after very large doses. Low blood values have also been reported in subjects with fatty and cirrhotic livers, alcoholism, alcoholic polyneuropathy and pellagra, pernicious anaemia, diseases of the central nervous system and Korsakoff's psychosis [728].

Excretion. The kidney concentrates vitamin B_1 from plasma to a marked degree, perhaps twenty times or more [705], although there is no direct relationship between urinary and blood levels. According to Harris and Leong [155], a negligible quantity only is excreted in the faeces, although this does not agree with the observations of Ritsert [172], who states that $180\mu\text{g}$ may be excreted daily in the faeces, and with the more recent work of Najjar and Holt [780]. They have conclusively shown that, in some individuals at any rate, bacterial synthesis can occur in the human bowel, and that there may be a considerable faecal excretion of vitamin B_1 even on a zero intake (p. 186). Harris and Leong conclude that in the metabolism of vitamin B_1 by the normal human body, about five to eight per cent. of the vitamin is quickly eliminated unchanged by the kidneys and a greater part of the remainder is presumably destroyed by metabolic changes in the tissues. Baker and Wright [158] state that on low vitamin B_1 diets the urinary excretion is 4.5 to 8 per cent. of the intake; on high diets high in vitamin B_1 ten to twelve per cent. of the intake.

The daily excretion of vitamin B_1 given by various investigators is given in the following table:—

Daily Excretion of Vitamin B_1 in Various Conditions

Author	Range of Excretion in micrograms	Average	Remarks
Benson, <i>et al.</i> [628]	92-602	268	Children on daily intake of $990\mu\text{g}$.
" " "	287-625	—	Children "saturated" with vitamin B_1 .
Goudsmit and Westenberg [166]	60-93	280	Men.
" " "	—	120	Women.
" " "	—	175	Daily intake $860\mu\text{g}$ vitamin B_1 .
" " "	—	15-50	Daily intake $260\mu\text{g}$ vitamin B_1 .
Harris and Leong [155]	72-105	—	Daily intake 1.26 mg.
Harris, Leong and Ungley [156]	86-105	66	Well nourished person.
" " "	12-36	24	Poor hospital patients.
" " "	—	10.5	Beriberi.
Melnick and Field [165]	90-198	—	Men.
" " "	—	—	Women.
Neuweiler [812]	trace to 26	5	Newborn.
Sciclounoff [159-160]	30-500	—	
Schroeder [162]	100-400	100-200	Normal subjects.
van Colvorden [168]	46-92	—	Pregnancy.
Wang and Harris [157]	90-480	150-240	Normal subjects.
Wang and Yudkin [167]	185-240	—	Daily intake 1 mg.
Werner [170]	100-260	—	Pregnancy.
Williams, <i>et al.</i> [827, 794, 795]	3-19	—	Intake of 0.1 mg. per 1,000 calories
" " "	10-42	—	Intake 0.22 mg. per 1,000 calories.
Wright and Baker [158]	120-540	180-240	Healthy subject.
" " "	40-66	—	Vitamin B_1 deficient diet.

If vitamin B₁ is injected intramuscularly the bulk is excreted in the next three hours [899]. The absorption is considerably delayed by the presence of 0.06 per cent. to one per cent. of zinc, which reduces the total urinary excretion in twenty-four hours by about half [784]. The absorption of numerous drugs, including insulin and adrenalin is delayed by zinc. The course of injected vitamin B₁ in the body has been studied by using synthetic vitamin B₁ made from radioactive sulphur, which can be detected in the faeces and urine by radioactive methods [701]. After injecting 16 mg. of radioactive vitamin B₁ into a normal subject it is found that after six days sixty-one per cent. of the radioactive sulphur is detected in the urine, eleven per cent. in the faeces, and twenty-eight per cent. cannot be accounted for. From this it is clear that the urine is the major excretory medium of parenterally administered vitamin B₁. Destruction of injected vitamin B₁ occurs rapidly in the body because neutral sulphur compounds and inorganic sulphate derived from the vitamin are found in the urine shortly after. Even after thirty-six days on a vitamin B₁ free diet it can be shown that some vitamin B₁ is still retained by the tissues.

Sciclounoff [159, 160] observed that the urinary excretion of vitamin B₁ for normal subjects is between 30 and 500 μ g per day. After the ingestion of 8 to 40 mg. of vitamin B₁, 5 to 35 mg. per cent. of the ingested vitamin is excreted. When 50 mg. was injected subcutaneously ten to twenty per cent. was excreted. According to Sciclounoff maximal elimination was not reached for one or two days, and for about a week after injections had ceased the urinary elimination remained at four to five times the normal level. The factors influencing the excretion of vitamin B₁ in any individual are unknown, and hence the clinical value of excretion studies on vitamin B₁ are of limited value, but Sciclounoff and Bauer [161] observed that the excretion of injected vitamin is considerably lowered in diseased subjects, who showed an excretion of four to ten per cent. A lowered excretion in diseased persons was also noted by Schroeder [162]. He gives the normal range as 100 to 400 μ g, with an average of 100 to 200 μ g per day. After the injection of 10 mg. intravenously varying proportions of the dose were excreted (8 to 4 mg.), but no constant relationship was found between the amount excreted before and after the dose. Drigalski [164] found that when the intake of vitamin B₁ was varied the excretion did not vary proportionately, and he considers that the method of estimating the degree of saturation of the body by measuring the excretion after large doses is of no practical value.

Experiments made by Melnick and Field [165] on normal subjects revealed that the excretion on a constant diet did not vary appreciably from day to day. When a test dose of 5 mg. vitamin B₁ was given with a large meal, twelve to fourteen per cent. was excreted within twenty-four hours. A reduction in the vitamin B₁ intake from 860 μ g to 260 μ g daily resulted in a drop in the urinary excretion from 175 μ g to 50 μ g within two days, subsequently reaching a value of 15 μ g daily. When a normal diet was resumed the excretion had not reached a normal level after fourteen days, but the proportion of a test dose excreted, though small during the period of low intake, returned to normal immediately the normal intake recommenced.

Wang and Yudkin [167] placed three volunteers on a controlled diet

deficient in vitamin B₁, administered graded supplements of the vitamin and measured the excretion by the modified thiochrome test (p. 284). They found that on a basal vitamin B₁ deficient diet the excretion fell rapidly to a steady value which was maintained for some time. A constant level of excretion was rapidly attained with each fresh level of intake. With increasing intake at first the excretion was raised only slightly, but when the intake exceeded 1.2 mg. the rise was more rapid. The average excretion on an intake of 1 mg. was 135 μ g to 240 μ g. Wang and Yudkin suggest that the excretion at lower levels is at the expense of the stores in the body and that the level at which excretion begins to rise rapidly gives some indication of the vitamin B₁ requirements of the individual. The percentage excretion at different levels varies from subject to subject, the variation no doubt being due to different rates of metabolism. According to Wang and Yudkin, a large increase of carbohydrate in the diet produces a lowering of vitamin B₁ excretion, an observation that Cahill [525] has been unable to confirm. He states that neither the fat nor the carbohydrate content of the diet affect the excretion of vitamin B₁. Wang and Yudkin suggest that an indication of the vitamin B₁ requirement can be obtained by noting the level of intake at which a rapid rise in excretion begins (cf. p. 240).

Williams and his co-workers [827, 794, 795] examined the urinary excretion of vitamin B₁ in subjects on diets deficient in the vitamin. On a daily intake of 0.1 mg. per 1,000 calories the daily excretion of vitamin B₁ fell to between 8 μ g to 19 μ g. After 120 days deprivation it fell as low as 8 μ g. Thirty days' treatment with vitamin B₁ was necessary to restore the urinary excretion to normal levels. On a daily intake of 0.22 mg. per 1,000 calories the daily excretion during a period of from 27 to 194 days fell to between 10 μ g to 42 μ g.

Similar studies on human volunteers placed on a vitamin B₁ intake of 0.86 mg. daily were made by Jolliffe and his collaborators [828]. They found that the urinary excretion of vitamin B₁ is roughly proportional to the dietary intake of the vitamin and that the urinary excretion reflects within twenty-four to forty-eight hours an increased or decreased vitamin B₁ intake. From thirteen to twenty-six per cent. of the ingested vitamin B₁ was excreted in a control period, and 7.4 to 28 per cent. during the deficiency period. Subjective signs of deficiency did not develop unless the vitamin B₁ excretion fell below 100 μ g daily. Objective signs, such as changes in the electrocardiogram, did not appear until the excretion fell below 30 μ g daily.

A study of the urinary excretion of vitamin B₁ in 100 pregnant women was made by van Coevorden [168], who found that it fell as pregnancy advanced. Thus the average figure at six months was 92 μ g daily, and this fell to 46 μ g at term. He considers an excretion of less than 50 μ g a day as a sign of hypovitaminosis B₁, and on this standard thirty-four per cent. of the women studied suffered from vitamin B₁ deficiency, and forty-two per cent. were on the border line. During the tenth month of pregnancy sixty-four per cent. were excreting less than 50 μ g daily. Higher figures, *e.g.*, from 100 μ g to 200 μ g a day are given by Gaetgens [169] and Werner [170]. Neuweiler [171] considers that the excretion in pregnancy is within the

same range as in normal women. Saturation tests, however, showed an increased tendency for pregnant women to retain a large proportion of an injected dose of 10 mg. vitamin B₁, especially if manifestations of pregnancy toxæmia were present.

It appears from the above observations that only a small proportion of the vitamin given by mouth or by injection is excreted in the urine, the rest being apparently destroyed in the body. The actual amount excreted depends upon the reserves or "degree of saturation" of the body tissues, but since it is water soluble these are soon used up. Vitamin B₁ is excreted mainly as such and very little as cocarboxylase, even if the latter is injected or given by mouth [426].

The excretion of vitamin B₁ in the urine is diminished in infections [786] and in aged persons [781], but is increased in thyrotoxicosis, probably because the thyrotoxic subject cannot store as much vitamin B₁ as a normal person because of the frequent co-existing hepatic and muscular disease [286]. The urinary excretion of vitamin B₁ is diminished during exercise, but returns to normal on resting [911]. Over short periods the administration of salicylates increases the excretion of vitamin B₁, but over long periods there is a reduced output [898]. The excretion of vitamin B₁ is considerably increased by the administration of mercurial diuretics such as mercuraphylline [914]. The increase is not due merely to increased diuresis, because the concentration of vitamin B₁ in the urine is several times greater than normal.

Several conflicting observations have been made on the excretion of vitamin B₁ in sweat. According to some observers appreciable quantities of vitamin B₁ can be lost in the sweat. Thus Hardt and Still [787] state that football players excrete twice as much vitamin B₁ in the sweat as in the urine, and that after large doses of vitamin B₁ the figure may be seventy times as much. They concluded that five to fifteen per cent. of ingested vitamin B₁ might be lost in the sweat. The average value recorded was 9 μ g per 100 c.c. Cornbleet and his co-workers [788] collected heat sweat by encasing subjects in a rubber bag and seating them in a cabinet heated sufficiently to obtain 100 to 200 c.c. of sweat in twenty to thirty minutes. This sweat contained 15 μ g per 100 c.c., equivalent to five per cent. of the intake of vitamin B₁ in a good diet. They concluded that the loss was negligible in normal subjects and only became significant in subjects working in a hot environment and doing heavy work, when the amount of sweat secreted might be as much as two or three litres daily. A much lower value, namely, 0.2 μ g per 100 c.c., is given by Mickelsen and Keys [789]. Other workers state that the amount of vitamin B₁ in sweat is either negligible or unmeasurable [790, 791].

PHARMACOLOGY

Vitamin B₁, like most other vitamins, exerts in addition to its metabolic effect a pharmacological action if given in relatively large doses. This may explain its therapeutic action in certain conditions in which there is no evidence of vitamin B₁ deficiency.

According to Hecht and Weese [185] subcutaneous injections of from

50–500 mg. per kg. of body weight cause an increase of twenty per cent. in the oxygen intake of guinea-pigs within half an hour, but Molitor and Sampson [186] state that vitamin B₁ only increases the oxygen consumption in B₁ deficient animals after food.

Vitamin B₁ possesses diuretic properties [185], and since the diuresis can be checked by barbiturates it is presumably of central origin [187]. Vasodilatation followed by a fall in blood pressure is said to follow the intravenous injection of very large doses in animals [186, 770]. Narcotised dogs show bradycardia and respiratory arrhythmia after receiving massive doses [189]. The vitamin in low concentrations increases the tonus of the isolated heart [721], but has a depressant effect on the small intestine and a stimulating effect on the tone of the large intestine, rectum and ureter [661]. According to Briem [574] it delays the onset of fatigue in the isolated muscle in Ringer's solution. Vitamin B₁ antagonizes the effect of nicotine on isolated smooth muscle, the effect being probably confined to the nerve synapses and end plates at the myoneural junction [920].

In the experimental animal it has been found that the ratio for the toxic dose when the vitamin is administered intravenously, subcutaneously and orally is 1 : 6 : 40. The symptoms of over-dosage in most animals studied include weakness, muscular tetany and spasm, slow and irregular breathing and finally respiratory failure. In experiments on monkeys Hecht and Weese [185] have shown that the ratio of the effective therapeutic dose to the minimum toxic dose is from 1 : 7,200 to 1 : 86,000. They also gave vitamin B₁ to rabbits over a prolonged period in doses of 50 mg./kg. a day intravenously, but the animals showed no loss of body weight and appetite, and their blood, blood pressure, urine and electrocardiograms showed no pathological changes. Similar results were obtained with rhesus monkeys.

Toxic effects are very rare when vitamin B₁ is given clinically, although they have been reported in sensitive individuals. Thus Steinberg [193] records that large intravenous doses produced herpes zoster in three patients, which disappeared in each case after the injections were stopped and returned when they were given again. The patients also suffered from nausea, epigastric fulness, a choking feeling and severe cramps. Mills [772] reported repeated instances of toxic effects from vitamin B₁ given in doses of 10 mg. to 50 mg. intramuscularly. Vomiting, involuntary voiding, collapse and temporary respiratory failure were experienced. In one patient the symptoms resembled those of hyperthyroidism. Laws, Schiff [722] and Eisenstadt [774] describe allergic symptoms following repeated injections of vitamin B₁ in doses of 25 mg. Angioneurotic oedema developed in one case five minutes after an injection; the symptoms were controlled with adrenaline. Other untoward symptoms that have been described are nervousness, flushing, tachycardia, profuse perspiration, giddiness, tinnitus, and a condition resembling anaphylactic shock, with a very high eosinophilia [668, 775]. Stein and Morgenstern [925] describe itching, sweating, dyspnoea, a tight feeling across the chest, and loss of consciousness after the subcutaneous injection of 100 mg. Most of these reactions would appear to be the result of sensitization as they occurred after several injections had already been given. It has been suggested

that an intradermal test be performed to determine sensitivity to vitamin B₁. Kalz [776] has shown, however, that an intradermal test is not conclusive proof of sensitivity as it gives a histamine-like wheal in normal persons.

These cases of intolerance to vitamin B₁ are comparatively rare. Williams and Spies [192] have given doses of 500 mg. daily for a month to patients without untoward effects and Jolliffe [777] has administered the vitamin to over 3,000 patients without a single mishap. The authors have not encountered a single case of intolerance over a period of seven years. There is some evidence, however, that intrathecal injection can cause severe reactions [572], and signs of meningeal irritation have also been reported [863].

HUMAN REQUIREMENTS OF VITAMIN B₁*

The estimates for the vitamin B₁ requirements of man are based on (a) analogy with the results of animal studies; (b) a study of the vitamin B₁ human content of diets; (c) the appearance or removal of the signs or symptoms of vitamin B₁ deficiency on graded intakes of vitamin B₁; (d) excretion studies on vitamin B₁ with or without a saturation test dose (p. 239) of the vitamin. Inferences from animal studies may lead to considerable error as different species differ in their vitamin requirements. With regard to the third and fourth methods, it must be remembered that the conclusions drawn from any one experiment or test are limited by the conditions of the test, and that other factors, such as sex, age, climate and metabolic activity may play a part.

Human Requirements based on Animal Studies. From a series of studies on different species of animals Cowgill [198] concluded that the vitamin B₁ requirement was related to calorie intake, and he arrived at the formula :—

$$\text{Vitamin B}_1 \text{ requirement} = K \times \text{weight} \times \text{daily calorie intake.}$$

For man this formula becomes :—

$$\text{Vitamin B}_1 \text{ requirement in I.U.} = 0.00142 \times \text{weight in kilograms} \times \text{daily calorie intake}$$

or

$$\text{Vitamin B}_1 \text{ requirement in mg.} = 0.00142 \times \text{weight in kilograms} \times \text{daily calorie intake} \times 0.008.$$

Applying this formula to an adult weighing 70 kg. and leading a moderately active life on 3,000 calories a day, the daily requirement would be 895 μg or approximately 0.9 mg. Cowgill's formula gives the minimal daily requirements of a normal adult, but it does not apply to infants, children and pregnant and nursing women. It gives no information on the optimal requirements which are much higher. It is suggested that fifty per cent. be added to Cowgill's predicted figure.

* Requirements are expressed throughout in milligrams or micrograms (μg). Where international units are given in the literature they have been converted.

Cowgill's formula may be rewritten :—

$$\frac{\text{Vitamin B}_1 \text{ requirements}}{\text{Calorie intake}} = K \times \text{weight.}$$

The B₁/calorie ratio may be taken as an index of the adequacy of vitamin B₁ intake. According to Cowgill the source of calories is unimportant. But studies by Arnold and Elvehjem [142] show that the fundamental relationship is not between vitamin B₁ and calories, but between vitamin B₁ and carbohydrate or non-fat calories. Since fat and protein are vitamin B₁ sparing (p. 168), the requirements of the vitamin are decreased on a high fat and protein/low carbohydrate diet, and increased on a high carbohydrate diet. The Cowgill formula is also challenged by Elsom and co-workers [786], who placed six volunteers on diets containing the amounts of vitamin B₁ predicted from the formula. Three developed signs of vitamin B₁ deficiency (p. 227); they were the smaller subjects of the group. The urinary excretion of vitamin B₁ was proportional to the intake, but was not related to body weight or calorie intake. The minimal intake sufficient just to prevent deficiency symptoms was 651μg daily, giving a vitamin B₁/calorie ratio of 0.85.

Arnold and Elvehjem [226, 227] calculated that rats, chicks and dogs can grow on a diet containing 80–100μg of vitamin B₁ per 100 gm. of low fat diet, and concluded that the human requirements could be calculated from this figure. In the case of a normal human diet, this corresponds to 0.5 mg. to 0.75 mg. daily. The fallacy of inferring the human needs of the various vitamins from animal studies has been pointed out.

Vitamin B₁ Requirements from Dietary Studies. The vitamin B₁ requirements of man have also been calculated from the dietary habits of human beings. In 1936 Baker and Wright [199] studied numerous dietaries, both past and present and from tables calculated the vitamin B₁ content, which varied from 600μg to 1.5 mg. daily. In a later paper with Drummond [200] they concluded that an intake of 900μg daily was a minimal quantity and that 1.5 mg. to 2.1 mg. was desirable. In criticism it may be pointed out that it is very difficult to predict the exact vitamin content of a diet from tables. Foods vary considerably in their vitamin content, which varies according to season, and locality, and according to whether the food is eaten raw or cooked. Losses of vitamin B₁ in cooking may be from as little as eight per cent. to as much as seventy-five per cent. (pp. 156, 157). Lane, Johnson and Williams [792] carried out a similar investigation, but in addition to computing the vitamin B₁ values of diets in various communities in America, they carefully analysed representative samples of food for the vitamin B₁ content. They estimate that the average vitamin B₁ intake for sixty-six per cent. to seventy-five per cent. of the population of America before the introduction of enriched flour or bread (p. 155) was 0.8 mg. per 2,500 calories. This is below the figure given by Stiebeling and Phipard [210], who made an extensive survey of the diets of families of workers in American cities, and concluded that all groups received more than 0.72 mg. daily. They consider that the *minimum* daily requirement of a 70 kg. man consuming 3,000 calories per day is 0.9 mg. On an average about half the families examined received 1.5 mg. or more daily, although

in some regional groups only one-fifth to three-fifths received more than 0.9 mg. daily. Five to twenty-two per cent. of city families spending \$1.25 to \$1.87 per head per week on food received less than 0.765 mg. daily. In Canada surveys show an even smaller intake. In Halifax, for example, all families examined had an intake of less than 1.05 mg. of vitamin B₁ per head daily, while the average per capita consumption was only 0.68 mg. In Edmonton seventy per cent. of the adults were receiving less than 0.9 mg. daily, and in Toronto fifty-seven per cent. received less than 1.05 mg. daily. Winters and Leslie [799] state that the daily intake may be as little as 0.49 mg. daily in persons of very low income in the United States.

A survey of the vitamin B₁ status of the population of Great Britain was made in 1940 by Moran and Booth [213], who concluded that about seventy per cent. of the population have an intake of 1 mg. or more of vitamin B₁ daily; ten per cent. receive only 0.68 mg. daily. They concluded that the national average is 1.18 mg. daily, and they consider that for optimum nutrition the desirable daily intake should be 2 mg. on a caloric consumption of about 3,000. If it is recalled that in diets of a hundred years ago wholemeal bread alone contributed between 1.5 mg. to 1.8 mg. of vitamin B₁ to the daily diet, an intake of 2 mg. a day is not difficult to achieve.

Elsom and Machella [727] determined the vitamin B₁ intake of a group of normal persons, who were permitted to select from an adequate diet the amount and type of food they required. The subjects were thus free from the desire to save money on food, a necessary practice in many families with low incomes, and the food was not spoiled by over-cooking. The average consumption of vitamin B₁ was 1.125 mg. daily. The individual intakes ranged from 1 mg. to 2.15 mg. daily. There was no correlation between the carbohydrate or caloric and vitamin B₁ intakes. This method would appear to be the most rational of any as it is not based on arbitrary blood or excretion tests, nor was the quantity and quality of the food limited to save money.

An Australian food survey suggests that the average adult in Australia has an intake of 0.84 mg. to 0.88 mg. of vitamin B₁ daily [212]. From a study of Eastern diets Jansen and Van Veen [207] estimate the average minimum vitamin B₁ intake as 0.3 mg. to 0.6 mg. daily. As beriberi and deficiency diseases are rife in the East such figures cannot be accepted as sufficient, particularly as beriberi has been reported on a vitamin B₁ intake of 0.4 mg. daily [228]. Meyers [728] had adduced evidence of adaptation to chronic low intakes of vitamin B₁ in studies of healthy Javanese labourers, and believes that man can adjust himself to an intake of 0.6 to 1 mg. a day. His criteria of adequate vitamin B₁ nutrition were based on excretion studies before and after a test dose. Meyers does not state, however, what standards he used for determining the health of the labourers, nor does he give the vitamin B₁ content of the diet before the test. Moreover, the size and weight, and therefore, basal metabolism of the Eastern male is lower than that of the European or American and, therefore, his vitamin B₁ requirements are presumably lower.

Requirements from Studies on Induced Vitamin B₁ Deficiency in Man.

Another method that has been used in determining the vitamin B₁ requirements of man depends upon the appearance or removal of the signs and symptoms of vitamin B₁ deficiency on graded intakes of vitamin B₁. It has been shown by several investigators that human volunteers subsisting on diets adequate in all factors except vitamin B₁ show a well defined deficiency syndrome (p. 227). This is characterized by easy fatigue, depression, irritability, failure to concentrate, muscle tenderness, poor appetite, E.C.G. and cardiac disturbances, polyneuropathy and constipation. Several investigators have produced these, and other manifestations, by giving very low intakes of vitamin B₁. The daily intake that just produces these manifestations or just relieves them is noted and taken as the minimal requirement of vitamin B₁. Williams and his collaborators [827, 715, 798, 794] have conducted a number of investigations along these lines (p. 227). They have kept volunteers on diets containing daily rations of vitamin B₁ varying from 0.2 mg. to 0.95 mg. and deficiency symptoms were noted in some individuals on all intakes between these two figures. Weight could be maintained but physical and mental efficiency were impaired. Their final conclusion is that at least 0.45 mg. of vitamin B₁ per 1,000 calories daily is needed to prevent deficiency symptoms [795]. This is approximately 1.25 mg. daily on an intake of 2,800 calories, which is the average figure for an adult male. Williams increases the requirements to 0.6 mg. per 1,000 calories to allow a safe margin.

Elsom and her co-workers [786] consider that a minimum daily intake of 0.65 mg. of vitamin B₁ will prevent deficiency symptoms. Williams and Mason [715] noted deficiency symptoms at intakes of this level (0.22 mg. per 1,000 calories), which disappeared when a diet providing 0.5 mg. per 1,000 calories was supplied. When the daily intake of vitamin B₁ was raised from deficiency levels to 2 mg. a day a sense of well-being with unusual stamina and enterprise was experienced [827]. Melnick [798] also observed deficiency symptoms on an intake of 0.26 mg. a day.

Foltz, Barborka and Ivy [981] kept four medical students on a restricted diet for nine to twelve months. On a diet of 0.2 mg. vitamin B₁ per 1,000 calories (approximately 0.5 mg. daily) they experienced definite objective and subjective signs and symptoms of dietary deficiency in two months. Tenderness and pain in the leg muscles and decreased appetite were observed on an intake of 0.38 to 0.38 mg. per 1,000 calories. On an intake of 0.17 to 0.21 mg. per 1,000 calories a decrease in appetite, diminished work output, muscle tenderness and pain, feelings of fatigue, deterioration in mental attitude and alertness developed after four months. Foltz, Barborka and Ivy consider that the minimal intake is 0.38 to 0.45 mg. per 1,000 calories or approximately 1 to 1.35 mg. daily.

These observations are not in agreement with those of Keys, Henschel and co-workers [796, 797, 980]. In their first study Keys and Henschel [797] could find no physiological reason for increasing the vitamin B₁ intake of soldiers above 0.48 mg. per 1,000 calories, or about 1.3 mg. daily. In a later study Keys and his co-workers [796] concluded from observations on a group of young men between eighteen and thirty that no benefit was obtained by increasing the daily intake of vitamin B₁ beyond 0.23 mg. per 1,000 calories. The volunteers were put on

diets providing intakes from 0.28 to 0.68 mg. per 1,000 calories daily. All subjects adhered to a fixed régime of physical work, and all conditions were standardized. The test included simple strength tests, responses during brief exhausting work, prolonged severe work and psychomotor tests of speed and co-ordination. Glucose tolerance, E.C.G. and X-ray studies were made and the heart rate, blood pyruvate, lactate, glucose and hæmoglobin were estimated at rest, during work and in the recovery period. Provided the intake of vitamin B₁ was not less than 0.28 mg. per 1,000 calories, muscular, neuromuscular, cardiovascular, psychomotor and metabolic functions were all normal. Clinical signs, subjective sensations, state of mind and behaviour were in no way affected. No conclusions can, of course, be drawn from these observations as to requirements over long periods, or for women, children and the aged, and in conditions which alter the human requirements of vitamin B₁. The discrepancies between the observations of Williams and Keys may be due to the fact that Williams' studies were carried out over periods up to six months, while those of Keys only took three months. If the intake is just less than the minimum daily requirement and the stores of vitamin B₁ are adequate, many months may be required to demonstrate clinical deficiency; hence the necessity of long term investigations. Studies on human requirements of vitamin B₁ will also require interpretation in the light of Najjar and Holt's observation that some human beings can synthesize vitamin B₁ in the gut [780].

Requirements based on Urinary Excretion Studies. Vitamin B₁ is excreted in the urine. The urinary excretion reflects the intake just before the time of the test, but it bears no relation to the vitamin B₁ level of the blood. Harris and Leong [206] concluded from excretion studies on normal healthy persons that the minimum daily requirement was 0.6 mg., with 0.9 mg. as an optimum. Widenbauer and Wieland [208] gave a volunteer a low vitamin B₁ ration until no vitamin B₁ was excreted in the urine and the blood level fell. Then the vitamin was given until it was excreted in the urine and the blood level approached the normal figure. The amount required daily to do this was 0.87 mg. to 0.55 mg. These earlier estimations were on the low side. More recently Melnick [782, 798] has used vitamin B₁ balance studies on a hundred and seventy five persons to determine the human requirements of vitamin B₁. In the deficient subject vitamin B₁ is conserved to replenish depleted tissue stores and not excreted to an appreciable extent in the urine. The addition of extra vitamin B₁ to the diet does not lead to increased excretion for some time. In the subject receiving an adequate supply of vitamin B₁ the urinary excretion is fairly constant and rises promptly after a test dose of the vitamin. Melnick [798] found that on a diet furnishing 0.86 mg. of vitamin B₁ daily the urinary excretion was constant, but fell precipitously when the intake was reduced to 0.26 mg. This produced clinical signs of deficiency (p. 227). According to Melnick a daily intake of 0.85 mg. of vitamin B₁ per 1,000 calories is just sufficient to saturate the subject so that there is a prompt response in the urinary excretion of the vitamin after a test dose of 5 mg. The intake recommended by Melnick is 0.5 mg. per 1,000 calories. Actually only seventy-five per cent. of those examined by Melnick were receiving

an adequate intake of vitamin B₁, although many of them were hospital staff and personnel, and could presumably afford diets containing sufficient vitamin B₁.

- Williams and his collaborators [794, 795] consider a urinary excretion of $100 \pm 10\mu\text{g}$ or more of vitamin B₁ in twenty-four hours, or a urinary excretion of 20 ± 2 per cent. of a test dose of 1 mg. given intramuscularly, evidence of adequate vitamin B₁ nutrition. They base this on the fact that these figures were obtained with volunteers receiving a daily intake of 0.4 to 0.45 mg. per 1,000 calories and that this was sufficient to prevent deficiency symptoms. They consider that 0.4 to 0.45 mg. per 1,000 calories is the minimum daily intake of vitamin B₁.

Human Biosynthesis of Vitamin B₁. Views on the human requirements of vitamin B₁ may require modification in the light of the observations of Najjar and Holt [780], who appear to be the first to have devised completely synthetic diets in studies on human vitamin deficiency. They gave nine adolescent males a synthetic diet, adequate in all respects, but lacking in vitamin B₁, which was added in graded amounts, beginning with 1 mg. daily. It was gradually reduced to between 0.1 mg. and 0.2 mg. and kept at this level for months. Contrary to expectations based on the work of previous observers (p. 227) no deficiency symptoms appeared. Finally vitamin B₁ was omitted from the diet altogether. In the course of three to five weeks only five of the nine volunteers showed evidence of vitamin B₁ deficiency. It was found that those subjects who had clinical manifestations of vitamin B₁ deficiency had practically no vitamin B₁ in their stools, while those who were symptom-free had large quantities, in spite of a zero intake. It was shown that vitamin B₁ was being synthesized in the large intestine by giving succinylsulphathiazole, which is bacteriostatic in this organ, to one of the subjects of the test; the vitamin B₁ content of the faeces fell to zero and returned to its former level when the drug was discontinued. Absorption of vitamin B₁ from the large intestine was proved by giving a retention enema containing the vitamin and showing a rise in the urinary excretion.

These observations demonstrate that the biosynthesis of vitamin B₁ can occur in man, although to what extent is not known. This would explain the discrepancies noted by various investigators working on the problem of human requirements. It also raises the question, does man require an exogenous source of vitamin B₁? Apparently some individuals can exist on very low intakes without developing deficiency symptoms [780, 796, 797]. It may be that the vitamin B₁ requirements of man cannot be sustained for an indefinite time by bacterial synthesis, which may only operate when the oral intake is very low. It is also possible that minute amounts of vitamin B₁ are needed for the growth of bacteria in the large intestine.

Human Requirements of the Adult. Summarizing the results obtained by the various methods described on pp. 181-186, it would appear that the minimum daily intake of vitamin B₁ is 1 mg. since deficiency symptoms have been recorded on intakes just below this. This is a minimal figure, the smallest amount for the performance of tissue respiration at normal rates. To allow for loss in the cooking of food and a margin for increased

requirements in response to special conditions it would seem wise to increase this to 2 mg. Even more may be required by a heavy worker or by a nursing mother. The actual requirement will depend upon the non-fat calories consumed and will, therefore, depend upon occupation, metabolic rate and sex among other factors. A minimum daily intake of 1 mg. per 8,000 calories was the recommendation of the League of Nations Committee on Nutrition in 1939 [211]. The Committee on Food and Nutrition of the National Research Council, U.S.A. (1942), recommend the following daily allowances, which can be met by a good diet of natural foods [800]:

	Calories	Vitamin B ₁ mg.
Man (70 kg.) :—		
Moderately active . . .	8,000	1.8
Very active . . .	4,500	2.8
Sedentary . . .	2,500	1.5
Woman (50 kg.) :—		
Moderately active . . .	2,500	1.5
Very active . . .	8,000	1.8
Sedentary . . .	2,100	1.2

Requirements of Infants and Children. From the data available in 1942 the Committee on Food and Nutrition of the National Research Council, U.S.A., suggest the following optimal allowances of vitamin B₁ from infancy to adolescence :—

	Calories	Vitamin B ₁ mg.
Under 1 year . . .	100/kg.	0.4
1-3 years . . .	1,200	0.6
4-6 " . . .	1,600	0.8
7-9 " . . .	2,000	1.0
10-12 " . . .	2,500	1.2
Girls, 13-15 " . . .	2,800	1.4
16-20 " . . .	2,400	1.2
Boys, 13-15 " . . .	3,200	1.6
16-20 " . . .	3,800	2.0

The needs of infants increase from month to month. The amounts given in the table for infants under one year are for approximately six to eighteen months. As the metabolic rate of the infant and child per unit of body surface is greater than that of the adult, a relatively greater requirement of vitamin B₁ would be expected.

The vitamin B₁ requirements of the infant have been calculated from the vitamin B₁ content of mothers' milk. On this basis Cowgill [218] suggests 0.24 mg. daily as a minimum for the young baby; Price [219] gives a higher figure, namely, 0.6 mg. Slater and Riall [759] calculated that a nursing mother excretes in her milk 0.15 mg. of vitamin B₁ daily, and they consider that 0.1 mg. is sufficient for the daily requirements of the infant on the basis of an intake of 284 non-fat calories daily. This would appear to be much too low. Knott and her co-workers [801] have carried out a number of investigations on the vitamin B₁ content of mothers' milk, which they state contains from 0.6 μ g per 100 c.c. in colostrum to 36 μ g in milk, with an average of 15 μ g. Examination of the milk of thirty-three healthy nursing mothers gave an average figure of 20 μ g of

vitamin B₁ per 100 c.c. The vitamin B₁ excreted in the urine of the infants was estimated and showed little difference after a test dose of vitamin B₁. It was concluded that young infants have a minimal vitamin B₁ requirement of 0.2 mg. daily, a quantity that can just be met by the normal healthy infant if the mother's milk contains 20 μ g or more per 100 c.c. Knott suggests that a daily intake of 40 μ g per kilo of body weight may be a practical standard to cover the ordinary needs of the young infant. Cows' milk contains more vitamin C than human milk. According to Knott [802] boiled, pasteurized milk contains 18 μ g to 35 μ g per 100 c.c., with an average of 24 μ g.

The vitamin B₁ requirements of infants have also been calculated from excretion studies. Using this method Widenbauer and Krüger [220] concluded that breast-fed infants had an intake of 9 μ g of vitamin B₁ per 100 calories, and that those artificially fed received 16 μ g to 17 μ g per 100 calories. Assuming a daily calorie intake of 400, the corresponding requirements would be 36 μ g and 64 μ g to 68 μ g. Both these figures and those given by the League of Nations Technical Commission, 1939 [211], namely, 30 μ g to 45 μ g per 100 calories, are lower than the more recent estimates of Knott [711, 801, 802]. Knott studied the urinary excretion of twelve infants, aged from one to six months, with intakes ranging from 60 μ g to 0.9 mg. of vitamin B₁ daily. When the intake reached 0.24 mg. or more daily, there was a marked rise in the excretion of the vitamin. She therefore concluded that an intake of 0.24 mg. daily meets the infant's immediate needs. This figure agrees with that obtained from the examination of the vitamin B₁ of mothers' milk. The optimum figure is given as 0.3 mg. to 0.4 mg. daily.

Another approach was made by studying blood cocarboxylase levels in infants. In the adult blood levels of 1 μ g or below per 100 c.c. are associated with deficiency symptoms—muscle cramps, muscle weakness, and fatigue. The optimum level in the adult is 5 μ g per 100 c.c. according to Knott. She found that intakes of 0.2 mg. of vitamin B₁ daily could not maintain a blood level of 5 μ g of cocarboxylase in the infant. This would suggest a daily intake of at least 0.2 mg. of vitamin B₁ for the infant. Schlutz and Knott [808] noted that an improvement in appetite occurred if the vitamin B₁ intake of the infant was raised from 36 μ g–45 μ g to 54 μ g–60 μ g per kilo of body weight. The latter figure for the newborn becomes 0.2 to 0.25 mg. ; at six months it is 0.35 to 0.45 mg.

Up to adolescence the vitamin B₁ requirements increase with age. Stiebeling and Phipard [210], as a result of their extensive nutritional survey in America, consider that children of one to five years require 60 μ g to 75 μ g per 100 calories, which on the basis of a daily intake of 1,800 calories corresponds to 0.8 mg. to 1 mg. daily. Similar figures were deduced by Knott [221] from vitamin B₁ retention studies for children of four to seven years. According to the Technical Commission of the League of Nations, children from three to five years require an allowance of 0.6 mg. to 0.75 mg. of vitamin B₁ daily.

Benson and his co-workers [804] studied the twenty-four-hour vitamin B₁ excretion in a group of children aged four to ten. The amount of vitamin B₁ excreted was proportional to the amount ingested, and was

approximately twenty-five per cent. of the latter. The vitamin B₁ content of the diet was considered adequate if twenty-five per cent. of it was excreted in the urine. Using this criterion it was concluded that the vitamin B₁ requirement of children of this age group is 0.99 mg. daily.

The vitamin B₁ requirement increases in childhood and adolescence roughly in proportion to the calorie requirement until the age of sixteen to twenty, when in the case of males the requirements are slightly more than those of the adult. Stiebeling and Phipard [210] state that males between sixteen and nineteen require 1.8 mg. of vitamin B₁ daily. The allowance given by the National Research Council, U.S.A., is 2 mg.

To summarize, the vitamin B₁ requirements in infants, children and adults vary with age and calorie intake. To cover the needs of all adults an optimum intake of 60 μ g to 75 μ g per 100 calories should be aimed at. Young children require more in proportion to their weight. These figures do not take into account the possible biosynthesis of vitamin B₁ in man (p. 186).

Requirements in Pregnancy and Lactation. It is generally considered that the vitamin B₁ requirements are increased in the pregnant and nursing woman. This is confirmed by the observation of Evans and Burr [214] that a rat suckling her litter requires five times as much vitamin B₁ as normally. There is complete disagreement, however, as to how much more vitamin B₁ is required by the pregnant and nursing mother. The following values are given by different authorities :—

Authority	Daily Requirement in Pregnancy and Lactation
Baker and Wright, 1936 [199] . . .	8.5 \times minimum = 8.5 mg.
Cowgill, 1938 [198] . . .	45 μ g to 60 μ g per 100 cal. = 1.125 mg. to 1.5 mg.*
League of Nations, 1938 [216] . . .	2 to 3 \times minimum = 2 to 3 mg.
Williams and Spies, 1938 [192] . . .	5 mg.
Dieckmann and Swanson, 1939 [563] .	First 28 weeks, 1.2 mg. Last 12 weeks, 1.8 mg.
Stiebeling and Phipard, 1939 [210] .	1.5 mg.
Gahtgens, 1940 [564] . . .	1 mg.
Williams, <i>et al.</i> , 1940 [565] . . .	1.4 mg.*
Toverud, 1940 [806] . . .	4 to 5 \times minimum = 4 to 5 mg.
Shute, 1942 [807] . . .	4 \times minimum = 4 mg.
National Research Council, U.S.A., 1942	
Neuweiler, 1943 [808] . . .	2 mg.
Lockhart <i>et al.</i> , 1943 [809] . . .	3 \times minimum = 3 mg.

* Based on a daily intake of 2,500 calories in pregnancy.

Some of these figures are based on dietary studies and surveys and others on excretion studies. Earlier workers noted that there was a large retention of a test dose of vitamin B₁ in pregnant women [171, 263], and others [565] noted that pregnant women on intakes of lower than 45 μ g per 100 calories showed deficiency symptoms such as nausea, vomiting, fatigue and paresthesiæ, and abnormal changes in the electrocardiogram. Toverud [806] noted that forty-six per cent. of a group of 114 pregnant women excreted practically no vitamin B₁, and eight out

of ten none after a test done of 5 mg. It was necessary to administer to pregnant women 4 mg. to 5 mg. of vitamin B₁ daily to obtain an excretion corresponding to normal non-pregnant controls. The requirements of the pregnant woman were therefore considered to be 4 mg. to 5 mg. daily, and higher still in lactation. Lockhart and his colleagues [809] found the amount of vitamin B₁ necessary to produce a urinary excretion peak in normal controls. The amount required by pregnant and nursing women was three times as much. Shute [807] noted that the incidence of deficiency neuritis in 957 gynaecological cases was four per cent., and in 580 obstetrical cases, seventeen per cent. By a curious method of reasoning he therefore assumes that the requirements of vitamin B₁ in pregnancy are four times the normal.

According to the investigations of Knott [801] the nursing may get 0.4 mg. of vitamin B₁ daily in its milk. Allowing for the vitamin B₁ excreted in human milk, accepting Williams' optimum figure of 0.6 mg. per 1,000 calories in the adult, and an allowance of 3,000 calories for the nursing woman, it seems reasonable to conclude that she requires approximately 2.2 mg. of vitamin B₁ daily. This is almost the same as the figure 2.3 mg. accepted by the National Research Council, U.S.A. There is no reason why the pregnant woman should require much more vitamin B₁ than a very active woman. Her calorie requirement is 2,500 daily. A non-pregnant woman of 70 kg. is calculated to require 1.5 mg. of vitamin B₁ on a 2,500 calories diet. Allowing for the increased metabolism and body weight in pregnancy it may be assumed that the pregnant woman's requirements are between 1.5 mg. and 2 mg. daily. Evidence that the pregnant and nursing woman requires much more than this, *e.g.*, from 8 mg. to 5 mg. daily, is based mainly on excretion studies in which arbitrary techniques and assumptions are used. Unless supplements of synthetic vitamin B₁ are taken, it is difficult to obtain more than 8 mg. of the vitamin daily in the diet, and it is reasonable to suppose that the vitamin requirements of man can be met by the diet alone.

Summarizing, it may be said that the vitamin B₁ requirements of the pregnant woman are met by an intake of approximately 2 mg. daily. In lactation with its increased metabolism and excretion of vitamin B₁ in milk, slightly more than this is required, *i.e.*, approximately 2.5 mg. daily. Due allowance should be made for losses of vitamin B₁ that may occur in the cooking of food.

Requirements in Relation to Special Conditions. The vitamin B₁ requirements are increased in conditions associated with an increased metabolic rate and with defective intake, absorption or rapid excretion of the vitamin. These conditions are further dealt with on pp. 207, 211.

In both experimental and clinical hyperthyroidism [120, 121, 222, 296] and during exercise [197] the vitamin B₁ requirements have been found to be increased. It is generally agreed that in febrile and toxic conditions the vitamin B₁ requirements are increased, and this view has been confirmed by the beneficial effects observed from administering additional quantities of the vitamin when high calorie diets are being fed to patients suffering from prolonged pyrexia. It is suggested that in such cases the patient's requirements of vitamin B₁ be calculated on the calorie intake. The

vitamin B₁ requirements are also increased in heavy muscular work, rapid growth and delirium.

Mills [725, 892] has shown that the optimal vitamin B₁ requirements for rats is twice as high at an environmental temperature of 91° F. as at 65° F. The provision of additional vitamin B₁ also protected the rats against the severe effects of excessive heat.

Vitamin B₁ may be lost through the excretory channels in certain conditions, so although the patient's actual intake may be sufficient in theory, the amount remaining for use in the tissues may be quite inadequate. Thus diarrhoea is a common symptom of many clinical conditions, and Dann and Cowgill [228] showed in experiments on dogs that moderate diarrhoea decreases the absorption of a considerable quantity of the ingested vitamin by hastening the food through the small intestine. In some cases an additional fifty to seventy per cent. of the vitamin had to be administered to prevent the onset of anorexia. It is clear that in the more serious diarrhoeas even greater losses may be expected. The loss may be counteracted by the administration of additional vitamin B₁, preferably parenterally.

Vitamin B₁ is excreted through the kidney, and serious losses of the vitamin from the tissues can occur as the result of excessive diuresis. Cowgill [224] and his co-workers administered large volumes of water by mouth to dogs, and the vigorous diuresis produced resulted in the appearance of symptoms of vitamin B₁ deficiency. Control experiments showed that the administration of the same amount of water together with supplementary supplies of vitamin B₁ did not produce signs of B₁ deficiency. Diuresis occurs in many conditions, and in the treatment of many diseases large volumes of liquid are administered with the view to eliminating toxic substances, or reducing the possibility of genito-urinary infections. Animal experiments suggest the wisdom of administering additional quantities of vitamin B₁ (and vitamin C, which is also water soluble) in cases of diuresis resulting from disease or treatment.

Borson [414] found an increased vitamin B₁ requirement in diabetes. This may be associated with perverted carbohydrate metabolism or with the diuresis associated with diabetes.

An analysis of many hospital diets shows that they contain well below the minimum intake of vitamin B₁ [679].

HUMAN DISEASE ASSOCIATED WITH VITAMIN B₁ DEFICIENCY.

BERIBERI

Incidence. The incidence of beriberi is greatest in those regions where polished rice and refined cereals form the bulk of the diet. Rice is the staple foodstuff of half the human race, and in India eighty to ninety per cent. of the total calories are supplied by rice. To provide sufficient vitamins, the rice must be supplemented with maize, legumes or fish. When the supply of these fails, the incidence of beriberi increases. It is endemic in Japan, Southern China, the Philippines, East Indies, Malay Peninsula, and Southern India. The disease is said to be the eleventh

most common cause of mortality in Japanese infants, and a recent survey of sickness in the Japanese army revealed that thirty-nine per cent. of the cases were due to nutritional diseases, mainly beriberi. A fifth of all disease in Malaya is attributed to beriberi. In the years 1920-29 there were on the average 17,000 deaths from beriberi annually in Japan. According to a report by Fehily [680] eighteen per cent. of the admissions to the Infant Welfare Centre in Hong Kong show clinical signs of beriberi. The disease also occurs sporadically in small outbreaks on board ship, among beleaguered troops, *e.g.*, at the Siege of Kut in 1916, and in prisons and mental asylums. It has been reported prevalent in Labrador and Newfoundland, where white bread, molasses and salt meat form the major part of the diet of many of the inhabitants. The condition of frank beriberi as met with in the East is practically unknown in England, although a few cases which develop among seamen may find their way to English ports. Yudkin [228], who made a search of the literature in 1938, failed to find a single reference to true beriberi reported in England. A case which he observed himself was an Indian who had come to England from Calcutta. The patient was estimated to be on a diet containing 0.4 mg. of vitamin B₁ daily. Palmer [245] gives details of an English girl suffering from anorexia nervosa, who had been on a diet deficient in vitamin B₁ for some years, and who was originally diagnosed as a mental case. Careful examination showed that she was a mild case of beriberi. An English case of "Rand scurvy"—a combined vitamin B₁ and C deficiency—is described by Young [678]. Balfour and Tolpade [557] state that every pregnant woman in South India suffers from beriberi. The extremely high maternal mortality in these women is caused by a macrocytic type of anæmia which is quickly improved by yeast, liver extract, and a meat diet, all of which are rich in the vitamin B complex.

Ætiology. It has been generally supposed that beriberi is a deficiency disease due to lack of vitamin B₁. Deficiency diseases, however, are never limited to lack of a single factor, and although there is a vitamin B₁ deficiency in beriberi the disease is undoubtedly a multiple deficiency syndrome. Because the œdema and cardiovascular symptoms of beriberi respond dramatically to pure vitamin B₁ it has been argued that beriberi is therefore a disease specifically associated with a deficiency of this vitamin. It is possible that in beriberi the graver signs and symptoms of vitamin B₁ deficiency, which are neurological and cardiovascular, manifest themselves before those due to other deficiencies. When pure synthetic vitamin B₁ became available it was found to produce a rapid cure of certain manifestations of beriberi, but some persisted or even grew worse. It is now recognized that the treatment of beriberi is more satisfactory with vitamin B₁ and foods or concentrates rich in the B complex than with pure vitamin B₁ alone. Recent observations on induced vitamin B₁ deficiency (p. 227) have also shown that it is impossible to produce beriberi experimentally by diets poor in vitamin B₁ only. Polished rice, which is the staple food where beriberi is endemic, is not only deficient in vitamin B₁, but also in vitamins A, D, E and B₆, riboflavin, nicotinic acid, pantothenic acid, choline, calcium and iron [196]. A deficiency of vitamin A and riboflavin in animals has been shown to produce degenerative changes

in the spinal cord and peripheral nerves (pp. 34, 320). Does a deficiency of vitamin E contribute, in part, at any rate, to the advanced muscular degeneration seen in beriberi? Does lack of essential amino acids play a part? No selected combination of foods is entirely lacking in one vitamin. Clinically few patients present all the classical signs attributed to any single avitaminosis; actually any one case if carefully examined shows those of several. Certain manifestations are common to beriberi and pellagra, *e.g.*, weakness, nervous irritability, vague malaise, lassitude, mental confusion, depression and inability to concentrate.

It has been shown that a condition resembling beriberi can be induced in monkeys on a diet partially deficient in vitamin B₁ [729]. Treatment of the animals with the vitamin led to improvement, but not to complete cure. When the monkeys were deprived of vitamin B₁ completely, death supervened before clinical symptoms or nervous degeneration occurred.

Williams [696] doubts whether vitamin B₁ deficiency alone is responsible for the classic features of beriberi (p. 195). Meiklejohn [698] and Walshe [704] have also questioned the view that vitamin B₁ is the specific anti-neuritic vitamin. While it is true that nerve tissue uses carbohydrate only for its metabolism, vitamin B₁ is necessary for the metabolism of all tissues and not for nervous tissue only. The possibility that man can synthesize vitamin B₁ [780] suggests that a lack of other factors is essential for the production of beriberi.

Certain Indian and Japanese writers have postulated a toxin as a cause of acute beriberi [805]. Japanese writers in particular believe infantile beriberi to be caused by a toxin in the mother's milk, because the infant recovers when taken from the breast. This toxin may be methylglyoxal, which has been found in the blood of patients with beriberi. Stannus [762], who accepts the toxin theory, suggests that the methylglyoxal is formed during the breakdown of carbohydrate in skeletal or heart muscle, and that in the absence of glutathione as a co-enzyme it cannot be broken down by glyoxalase. He postulates the possibility of a primary deficiency of glutathione in beriberi. Haynes and Weiss [699], however, were unable to produce the cardiac manifestations of vitamin B₁ deficiency by the injection of methylglyoxal, pyruvic acid or lactic acid.

It is not known for how long the diet must be depleted of vitamin B for the onset of beriberi, although it is stated that the symptoms appear after about three months on a poor diet [702]. Apparently an exclusive meat diet provides sufficient vitamins, as Steffanson and Anderson [280] existed on nothing but meat for several months without suffering from beriberi. Certain predisposing factors play a part in the development of the disease. These include poverty, increased physical exercise, infection, fever, hyperthyroidism, pregnancy, lactation, fatigue, dietary fads and idiosyncrasies, digestive disturbances and diseases interfering with the absorption of food, particularly gastric achlorhydria. Nixon [784] points out that many Chinese women appear normal in early pregnancy, but are often *in extremis* towards the end from beriberi. The frequent association of malaria and beriberi, observed particularly in Brazil and commented upon by Cowgill [198], is due to the heightened metabolism of malaria increasing the vitamin requirements.

Cases of beriberi resulting from inadequate vitamin absorption as a consequence of prolonged vomiting in such conditions as pyloric obstruction, prolonged diarrhoea, or short circuiting of the bowel have been reported. Loeb and Greenebaum [231] describe a case of wet beriberi resulting from impaired intestinal absorption due to a partial intestinal obstruction caused by a hernia of the mesentery. The diagnosis was confirmed by the improvement of the patient under vitamin B₁ therapy and by autopsy. Beriberi resulting from unbalanced diets, persistent vomiting, and alcoholism has been described [232-237].

Clinical Signs and Symptoms. Various clinical forms of beriberi have been described. Each case, however, presents individual variations, and mixed forms are seen.

Infantile Beriberi. This is common in the East among infants in the first few months of life and is characterized by a very rapid onset and

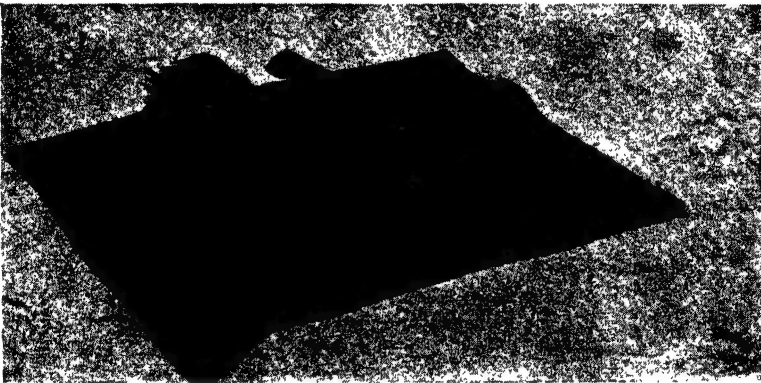


FIG. 86. Infantile Beriberi. The infant shows generalized œdema, and the body is held tense and rigid during a sudden paroxysm of pain.

acuteness, so that an apparently healthy child may die rapidly from the disease. This has given rise to the assumption, referred to on p. 193 that it is due to toxic metabolites [810]. Bray [811] gives a good clinical description of the condition. The infant suffers from anorexia and is disinclined to feed from the breast, milk is regurgitated, but water is not; it is restless and tender over the abdomen, particularly over the liver; abdominal distension is present and is accompanied by colicky pain, vomiting and paroxysmal screaming; constipation, diminished excretion of urine and water retention occur. The latter leads to œdema (Fig. 86) and an increase in weight, so that the infant looks plump, although wasting occurs later. There follows tachycardia (200 per minute), tachypnoea, dyspnoea and aphonia due to œdema of the larynx. The latter is responsible for the peculiar grunt or "beriberi" cry said to be characteristic of infantile beriberi. Later cyanosis, signs of cardiac enlargement and failure, congestion of the lungs and engorgement of the liver occur. The serous cavities and tissues become filled with fluid to produce a generalized œdema. Finally come signs of increased intracranial pressure with

meningism, rigidity, twitchings, drowsiness, coma and death. Each phase may only last a matter of hours and the whole condition a day or two. The sudden paroxysms of pain cause the body to be held tense and rigid (Fig. 86), although true convulsions do not occur. Infantile beriberi is rare in England, although it has been described on the Continent and may show an increase there during the present war period. The mortality in the East before the introduction of an extract of rice polishings (tikitiki) was very high (seventy-four per cent.), but this has been reduced considerably since the introduction of specific treatment. Post-mortem there is enlargement of the right ventricle and effusions into the pleural, pericardial and abdominal cavities. In addition there may be oedema or congestion of the liver, spleen and kidneys and oedema of the brain and lungs. Intercurrent disease often obscures the picture.

Women with manifest signs of beriberi may nurse infants having no apparent signs of infantile beriberi, and conversely seemingly healthy women may be nursing infants with manifest signs of the disease [810]. Such women and infants with no manifest signs are presumably in the latent stage. Congenital beriberi has been described, chiefly in the orient, although it has been reported in America [944]. The child is born with almost fatal cyanotic manifestations, aphonia, tachycardia and cardiac enlargement. Treatment of infantile beriberi consists of giving large initial doses of vitamin B₁, e.g., 50 mg. daily, and improvement of the diet of the mother. Fehily [810] states that the manifestations of vitamin B₁ deficiency are so common among the Chinese in Hong Kong that they are regarded as the physiological effects of child-bearing.

In the differential diagnosis of infantile beriberi the following must be considered: overfeeding, the results of which may resemble the vomiting of infantile beriberi; bronchitis, or bronchopneumonia, which are often complications of infantile beriberi; dyspepsia; meningitis; nephritis; peritonitis, which the gastro-intestinal syndrome of infantile beriberi may mimic; diphtheritic paralysis, which may be suggested by the dysphagia, aphonia and symptoms of circulatory failure in infantile beriberi; laryngismus stridulus, which may be suggested by the cyanosis and dyspnoea of infantile beriberi, although there is no "crowing" and Chvostek's sign is negative; congenital syphilis, which may be considered in view of the enlarged liver, oedema and loss of weight. Unless the diagnosis is entertained infantile beriberi may be easily overlooked in regions where the disease is not endemic.

The *adult or chronic type of beriberi*, in contrast to the infantile, is usually insidious in onset, except in cases of acute cardiac beriberi. Clinically the disease is characterized by a triad of symptoms—cardio-vascular disturbances, neuritis and oedema—and various forms are termed "dry" (neuritic, paraplegic), "wet" or "cardiac," according to the prevailing symptom. Neuritis is perhaps the most constant finding, although cardiac symptoms may be present. Early complaints are a feeling of fatigue, heaviness and stiffness of the legs with areas of paræsthesia and tenderness along the nerve trunks. Soon after the patient may notice headache, insomnia, anorexia, dyspnoea, tachycardia, nervousness and tenderness of the calf muscles on squeezing.

After a variable period the major symptoms manifest themselves. In *dry beriberi* (see Fig. 87) the nervous system is primarily affected. The clinical picture is one of ascending, symmetrical bilateral peripheral neuritis. Initially weakness and cramps in the legs are complained of, walking for short distances is unimpaired, but weakness is apparent after prolonged exertion (e.g., a mile walk), when the patient's legs will suddenly collapse under him. Later distances of a hundred feet may be sufficient to cause collapse. There is a burning sensation round the soles, and a numb-



FIG. 87. Dry or Paraplegic Beriberi. The arms and legs are chiefly affected. The leg muscles have atrophied and the patient exhibits a characteristic posture.



FIG. 88. Beriberi. Recovery from dry beriberi, showing hyperextension of the legs with posterior displacement of the tibia, due to laxity of the ligaments.

ness round the dorsum of the foot and ankle with a weakness in dorsiflexion of the ankle joint. The achilles and patellar reflexes are increased at first, then diminished and finally disappear. The ankle jerks are lost early, a sign of diagnostic importance. Weakness spreads upwards, first involving the extensor muscles of the foot, then the muscles of the calf, and finally the extensors and flexors of the thigh, which waste. Toe and foot drop can now be demonstrated. The affected muscles become tender, numb and hyperæsthetic, so that they are tender on palpation. Deep sensation, elicited by compression of the tendo achilles (Abadie's sign) is increased. The hyperæsthesia extends in the form of a band round the limb with

anæsthesia following in its wake ; loss of sensation over the tibia occurs early and is diagnostic. Atrophy of the muscles and skin follows, so that the limb has a wasted and shiny appearance. When tested electrically the muscles show the reaction of degeneration. Laxity of the ligaments of the knee joint may occur and give rise to hyperextension (Fig. 88). When the signs and symptoms are pronounced in the legs the upper extremities become involved, the hands and arms being affected first. Thomson [818] claims that neuritic symptoms may appear in the hands and arms before the legs. Burning, numbness and loss of power in the hands are experienced, followed by loss of tendon reflexes, wrist drop (see Fig. 89), hyper-



FIG. 89. Dry or Paraplegic Beriberi. Wasting of the extensors of the wrist and wrist drop.

æsthesia and anæsthesia. The grip becomes so poor that the sufferer cannot button his clothes or pick up small objects, and may find difficulty in feeding himself, although there is rarely paresis of the muscles of the face, tongue or pharynx. The gait becomes ataxic since the patient loses the power to raise the toes, and to avoid scraping them, he walks by lifting the hips, swings the legs, which are held wide apart (Fig. 87), and assumes a peculiar gait. That has been likened to walking in wet clothes or stiff clay. The ataxia is due to muscular weakness and not inco-ordination. As the disease progresses the patient becomes bedridden and suffers great pain from the pressure of the bed and clothes on tender muscles. Epicritic sensation is first affected, then temperature, pain and vibration sense. The muscles of the trunk and diaphragm may be involved, and muscular

contractures, and lack of muscular co-ordination may occur. Loss of sphincteric control does not occur until very late. Although mental symptoms are not common and generally speaking the psyche is clear, defects of memory often occur, and the tenth cranial nerve is sometimes involved. Months may elapse before severe symptoms occur, which may be precipitated by an infection or severe privation. Lesions of the optic nerve have been described [751, 753]. They include concentric contraction of the field of vision, temporal pallor of the disc and retrobulbar neuritis. These, however, may be due to a multiple vitamin B deficiency, and clear up after administration of the whole of the vitamin B complex.

Cardiovascular and respiratory symptoms predominate in acute *cardiac beriberi*, which is described as "wet" if oedema is present. Oedema is not seen in induced vitamin B₁ deficiency (p. 227) and may be due to lack of other essential nutrients, e.g., proteins, essential amino-acids or salts. The principal manifestations are dyspnoea, palpitation on exertion, precordial pain, tachycardia and oedema [241, 244]. This fulminating acute type, known as *shōshin*, or "acute pernicious" beriberi heart [241], is a serious threat to life, many patients dying suddenly if treatment is not instituted at once. The "beriberi heart" has long been known to clinicians working in the East. At any time death may occur suddenly from acute heart failure. The heart is enlarged both to the right and left, although mainly to the right, the liver swollen, tender and pulsating, the veins of the neck engorged, and the pulse small and thready. The carotids pulsate violently and pulsation is visible or palpable in the epigastrium and jugulars, a sign of tricuspid insufficiency. Breathing may be so laboured as to suggest respiratory obstruction. Systolic murmurs and signs of pulmonary congestion are common. There is a transient elevation of the systolic and diastolic blood pressures, and the electrocardiogram may show distinct changes (p. 222). It is of low voltage and there is an indefinite inverted or flattened T wave in leads I, II and III, shortening of the P-R interval, and prolongation of the Q-T interval. On palpation a bounding quality is noted in the larger arteries and "pistol shot" sounds may be heard on auscultation. Systolic murmurs and a loud sharp second sound over the pulmonary area may be heard. The heart sounds have also been likened to the beats of a pendulum clock, i.e., they are evenly spaced. The pulse quickens rapidly on exertion, and the pulse pressure is high because of a lowering of the diastolic pressure. The skin is usually warm, moist and of normal colour, and cyanosis is rare. Circulatory failure may be right or left sided. Weiss and Wilkins [242] examined a number of "beriberi hearts" in America, and in contrast to Eastern beriberi, the majority did not show signs of right-sided failure. Sudden circulatory collapse may occur.

Oedema is conspicuous in cases of "wet" beriberi (Fig. 40), beginning in the feet and legs and extending up the body to the face, eventually leading to ascites, hydrothorax and hydropericardium. This generalized oedema may mask the muscle wasting, and oliguria occurs while the oedema is developing. There are apparently no renal changes accompanying beriberi oedema, which is firmer than that of nephritis and of a hydrostatic nature, being due to the lowering of the tone of the arterial walls and

increased tension in the capillaries [237]. The rapid blood flow, warm extremities, flushed colour and increased arterial pulse pressure indicate a generalized arteriolar dilatation and an increased capillary pressure, which leads to oedema. The rapid blood flow returns the blood to the right side of the heart at an increased rate. The heart being weaker than normal fails to deliver the blood to the lungs as fast as it is received, and congestion of the viscera commences. If pulmonary oedema develops the right heart fails, its chambers dilate, and the acute beriberi heart results. There is precordial distress or pain, which is aggravated by food, so that the patient eats little. Gastro-intestinal symptoms such as anorexia, diarrhoea, and vomiting may be present. Aphonia is common, especially in infantile beriberi and in chronic adult cases. A mild secondary anaemia and sometimes hyperglycaemia appear in the picture, and amenorrhoea often occurs. Neuritic symptoms are often present. In fact, it is most probable that "wet" beriberi is "dry" beriberi with oedema [818].

Thomson [818] has seen deficiencies associated with lack of other vitamins in beriberi patients in the Straits Settlements. Thus he describes eczema of the scrotum, curable by yeast and marmite (*cf.* p. 335); cheilosis and corneal vascularization (pp. 329, 341), a red burning tongue and mouth responding to nicotinic acid; hemeralopia; and an anaemia curable by iron and marmite.

"Alcoholic beriberi" may be produced by chronic alcoholism, which leads to anorexia, chronic gastritis and so to deficient food intake. Cowgill [198] has pointed out that one of the first signs of vitamin B₁ deficiency is anorexia, which in turn leads to further diminution in vitamin intake. A vicious circle is set up. Alcoholic liquors supply a high calorie value with practically no vitamin B₁—an ideal combination for the development of beriberi. Weiss and Wilkins [242] described 120 cases of "beriberi heart," many of which were attributed to alcoholism. Generally speaking the cardiovascular symptoms predominate.

Diagnosis. The diagnosis of beriberi is made on a reliable dietary history, a careful physical examination, certain special tests, and the

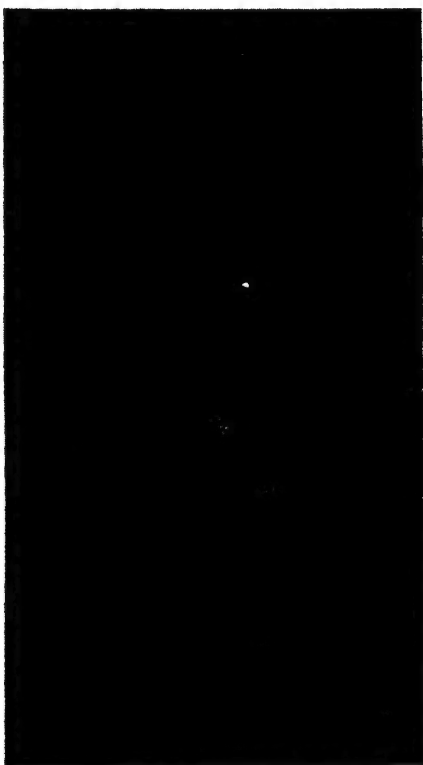


FIG. 40. Wet or (Edematous) Beriberi. Note the ascites, with obliteration of the umbilicus, and oedema of the legs, genitals and face.

therapeutic test of administering vitamin B₁. The dietary history reveals that the patient subsists on a diet abundant in over-milled rice, wheat or corn, or indulges in dietary idiosyncrasies, *e.g.*, white bread and tea. The history may also reveal that the patient is a victim of anorexia nervosa, alcoholism, or of some gastro-intestinal disease interfering with adequate absorption of water soluble vitamins (p. 209). Persons on a low vitamin B intake needing an increased requirement of the vitamin on account of pregnancy, lactation, hard physical exertion, or increased metabolism are also likely to suffer from beriberi. In the case of infantile beriberi the mother's dietary history, especially during the latter months of pregnancy, may be of diagnostic importance and signs of latent beriberi should be searched for in her.

The typical case of frank beriberi with an enlarged heart, peripheral neuritis, oedema, tenderness and atrophy of the muscles, and a history of dietary deficiency presents no difficulty, but the mild or latent case, which is more frequent, may be difficult to recognize. Early manifestations are dyspepsia, "pins and needles" in the extremities, fatigue, breathlessness, palpitation, and slight oedema round the ankles. Simple breath-holding and arm-raising tests will confirm the dyspnoea and fatigue.

In addition to peripheral neuritis the following neurological signs and symptoms are of diagnostic aid (Vedder [246]).

(a) Abadie's sign, *i.e.*, squeezing of the calf muscles. This causes pain.

(b) Areas of complete anæsthesia to pin-pricks may occur on the legs, especially over the anterior surface of the tibia.

(c) Loss of patellar and ankle reflexes.

(d) If the subject squats on his heels the position is very painful and he may be unable to rise without using his hands (*jongek* or squatting test).

(e) Characteristic shuffling gait.

(f) Aponia and the grunting "beriberi" cry are said to be characteristic of infantile beriberi.

The cardiovascular manifestations do not comprise an easily recognized syndrome. But their occurrence with signs of peripheral neuritis, dietary inadequacy, and the absence of syphilitic, hypertensive, and rheumatic heart disease, or of heart failure secondary to pulmonary disease, should suggest the possibility of beriberi. Other associated lesions are pellagroid dermatitis and glossitis [242]. It is not uncommon to find achlorhydria associated with beriberi heart [236].

Wenckebach [241] considers the following cardiac symptoms of value in the diagnosis of beriberi.

(a) Enlargement of the heart, especially to the right, as shown by percussion, auscultation and radiography.

(b) Murmurs, chiefly systolic, but also presystolic, with a resonant first sound. To this may be added a loud sharp second sound over the pulmonary area.

(c) Visible and palpable pulsation over the heart, especially to the left of the sternum.

(d) A bounding pulse and palpable pulsation over the large arteries.

(e) Distension of veins in the neck and arms.

(f) A painful and enlarged liver, with pulsation in severe cases.

(g) Right-sided failure, with pulmonary congestion as a terminal condition.

The oedema resembles a combination of that due to renal and cardiac insufficiency, since the extremities, abdomen, face and genitalia are affected (Fig. 40). The absence of albuminuria, the relatively high renal efficiency, and the normal protein content of the plasma exclude renal oedema. There is also less venous engorgement than would be expected from the extent of the oedema were it purely cardiac.

Special tests may be employed to confirm the diagnosis. (a) *Laboratory tests* for the detection of vitamin B₁ deficiency and their interpretation are described on p. 284.

(b) *Aalsmeer's adrenaline test* is considered to be of considerable help in diagnosis. It depends upon the rapid fall of diastolic pressure and the precipitation of typical heart attacks which follow the injection of adrenaline. An audible sound also develops in the antecubital fossa if not already present. The Aalsmeer test, which is striking in the acute cases and less so as the clinical condition improves, is not without danger, and must be employed with caution during the acute stages of beriberi. Apart from clarifying the diagnosis it is useful in following the progress of some patients. Pitressin is also used in place of adrenaline (*Wenckebach's test*).

(c) *Volhard's diuresis test* is considered to be of more value than Aalsmeer's or Wenckebach's tests. The fasting patient is given a litre of water and the volume of urine passed every half-hour is carefully charted for four hours. A normal person excretes all this fluid in this time, but in beriberi there is water retention. After treatment with vitamin B₁ the test should be negative, otherwise the findings are not considered significant.

(d) The combination of an increased minute output of the heart and an increased circulation time with the signs of congestive failure is an outstanding feature of the beriberi heart [242, 77]. Other causes of congestive failure, except hyperthyroidism, result in conspicuous slowing of the circulation.

(e) Electrocardiographic changes in the beriberi heart have been described by many investigators [286, 241, 242], but they are not constant, and indeed are not always present, even in cases with advanced heart failure. The changes when they do occur are reversed by vitamin B₁ therapy. The only feature of diagnostic importance appears to be a shortening of the P-R interval [241, 286]. The electrocardiogram is of value in diagnosis when the changes can be reversed by the administration of vitamin B₁ (Fig. 48).

Differential Diagnosis. Beriberi must be distinguished from peripheral neuritis due to other causes. In lead neuritis only the motor nerves and anterior horn cells are affected; sensation is rarely affected and pain is infrequent. The upper extremity is usually affected first (wrist drop). A history of colic, contact with lead, basophil punctation of the red blood cells, and the blue line on the gums help to establish the diagnosis. Peripheral neuritis due to other heavy metals resembles that caused by lead.

Peripheral neuritis due to external toxins is usually abrupt in onset,

and motor and sensory disturbances are common. Diphtheria produces an ascending paralysis beginning from the site of infection, *e.g.*, faucial diphtheria leads at first to palatal paralysis, only later spreading to contiguous parts of the nervous system. Arsenical neuritis perhaps mimics the polyneuritis of beriberi most closely. Here sensory changes, such as burning, pain, and paræsthesiæ are as marked as motor symptoms. The dermatitis of arsenical poisoning is, however, typical in appearance and distribution.

The cardiovascular manifestations may resemble those of heart failure due to other causes, *e.g.*, rheumatic, hypertensive, senile and syphilitic heart disease, and secondary to pulmonary disease. These can be distinguished by the history, clinical examination of heart and lungs, Wassermann reaction, and high blood pressure in the case of hypertension.

Other possible causes of œdema must be considered. In beriberi there is no albuminuria, in contrast to renal œdema. Severe anæmia is unusual in beriberi, although common in other forms of œdema associated with dietary deficiency. "Hunger œdema," which has been reported in many wars and famines, frequently occurs in underfed persons doing heavy manual labour. The œdema occurs in the feet and often in the face. In addition the patient complains of asthenia, polyuria, neuritis, and muscular weakness. Whether vitamin B₁ deficiency is a causative factor is unknown; it is generally considered that the principal factor is a low protein intake, which leads to lowered plasma protein concentration, and consequently to the flow of fluid into the tissue spaces.

Cases of beriberi have also been diagnosed as tabes dorsalis, muscular rheumatism, progressive muscular atrophy, malaria, ancylostomiasis and lathyrism. Tabes can be excluded by the Wassermann reaction, the Argyll-Robertson pupil, lightning pains, the characteristic sensory changes and by an examination of the cerebrospinal fluid. In both tabes and beriberi, however, Abadie's sign (p. 200) is positive. According to Sir Philip Manson Bahr true rheumatic disease is rare in areas where beriberi is endemic. Malaria, the most protean of tropical diseases, is differentiated by means of a stained blood film. Ancylostomiasis is characterized by anæmia, loss of reflexes but absence of paresis, eosinophilia, and the ova in the stools. In lathyrism (p. 74) there is no muscle tenderness or anæsthesia, and the knee-jerks are increased.

Pathology and Morbid Anatomy of Beriberi. The acute cases, which are often well covered with fat, die from heart failure, and the principal post-mortem changes are seen in the heart, with no macroscopic changes in the nervous system. The chronic cases seldom die from beriberi itself, but from complications, such as tuberculosis, typhoid, or some other intercurrent infection.

The nervous lesions are noted chiefly in the nerves supplying the lower extremities, *i.e.*, the sciatic nerve and its branches, but some degree of degeneration may be found in any peripheral nerve, the cranial nerves, and the vagus. The peripheral part of the nerve is the first to suffer. The nervous lesions cannot be recognized macroscopically. Histological examination reveals degeneration of the myelin sheath in the majority of the nerve fibres, with pigmentation and final vacuolar degeneration of the

cells of Schwann. The axis cylinders may show fragmentation or atrophy and Wallerian degeneration may be demonstrated, although as Spies and his co-workers point out, clinical signs of severe neuritis may occur and yet the myelin sheath may appear normal on microscopic examination [561]. As the disease progresses more axis cylinders are involved. Such changes are more advanced in the nerves of the legs, although they may also occur in the phrenic nerve and the vagus. There is also degeneration of the nerve cells of the sympathetic system, *e.g.*, in the cardiac, solar and renal plexus and splanchnic nerves.

The muscles supplied by the affected nerves are considerably atrophied, especially in the legs, thighs, upper extremity and diaphragm. Histologically there is evidence of cloudy swelling, fatty degeneration, loss of cross striation and shrinkage of sarcoplasm. Such changes are not characteristic of beriberi but of a severe polyneuritis. In "wet" cases oedema, with separation of muscle fibres, may be observed.

There are no gross changes in the central nervous system, although the brain, its membranes, and the spinal cord may be congested. Microscopically degeneration of the medullary sheath has been observed in many of the spinal tracts, in the posterior columns, and anterior and posterior nerve roots. The axis cylinders show signs of fragmentation and the ganglion cells show swelling of the cell bodies, peripheral displacement of their nuclei, and the Nissl granules undergo chromatolysis. Vedder [246, 247] describes lesions in the ganglion cells of the medulla and pons.

At post-mortem the heart is enlarged, the average weight of the heart of Japanese subjects dying from beriberi being 368 grams (normal 300 grams). The enlargement is particularly noticeable on the right side, especially the right auricle with a paper-thin wall, through which the dark semi-fluid blood within may be seen. The conus arteriosus, particularly at the site of origin of the pulmonary artery, is enormously dilated, a sign of diagnostic importance according to Wenkebach. The myocardium shows fatty degeneration and hydropic degeneration of the muscle and conductive fibres and an increase in the intercellular substances. In vitamin B₁ deficient rats cardiac dilatation due chiefly to dilatation of the right auricle is seen at autopsy. Atrophy and necrosis of the muscle fibres are visible on microscopic examination [910]. Owing to the right-sided heart failure, back pressure causes congestion of the liver, kidneys, spleen and intestines in a number of cases of beriberi. The "nutmeg" liver is common. Petechial hæmorrhages are observed beneath the pleura, and in the stomach and duodenum, while the vessels are hyperæmic. Internal organs are congested and may show fatty degeneration and cloudy swelling. The cause of the right-sided hypertrophy and dilatation is not definitely known. There is generalized arteriolar or capillary dilatation, which results in an increased flow of blood to the right side, which may be responsible for the hypertrophy and dilatation.

Oedema of the lungs is often found as well as serous effusions of a clear greenish yellow colour in the pericardium, pleura and peritoneum. Legs, thighs and arms may be oedematous in the "wet" form of beriberi. Various explanations of the oedema have been put forward, but the most

likely one is that it is due to generalized capillary dilatation with increased permeability to plasma.

The endocrine glands usually show post-mortem changes. Thus the pancreas is shrunken and sometimes cirrhotic, although the islets of Langerhans are sometimes hypertrophied. The adrenals, pituitary gland, and thyroid are usually enlarged. Robertson, Bard and Chen [815] describe thickening and gelatinization of the gall bladder.

The diagnosis of beriberi post-mortem rests on (a) dilatation and hypertrophy of the right side of the heart without evidence of organic cause, (b) congestion of the viscera, (c) œdema, (d) degeneration of peripheral nerves, and (e) absence of any other cause of death.

Prognosis and Treatment. The course of the disease is usually progressive unless treated, and sudden death may result from heart failure or from secondary infection. The mortality varies from five to fifty per cent. according to the severity and the treatment used. If treatment is given early enough complete recovery may occur, though regeneration in the nervous system may be slow. The mortality rate is high in the acute cases with cardiac involvement. Once remission has occurred the prognosis is good if the patient is placed on a well-balanced diet rich in vitamin B₁ and the B complex.

Evidences of grave heart implication, such as pulsation of the vessels of the neck, cardiac enlargement to the right, a rapid feeble pulse, cyanosis, dyspnoea and dilatation of the stomach are unfavourable and demand instant treatment. Diaphragmatic paralysis, serous effusions, oliguria and paralysis of the intercostals are also serious.

For prophylaxis a well-balanced diet is all that is requisite. Foods rich in vitamin B₁ and the B complex, such as whole cereal grains, peas, beans, lentils, ground peanuts, and yeast or yeast extract are useful adjuncts to the diet, and should be considered by those feeding armies in the field, institutions, isolated colonies and populations in tropical countries. In the East use has been made of an extract of rice polishings known as tikitiki. Particular attention should be directed towards the diets of persons with chronic debilitating diseases and increased metabolism, since the incidence of beriberi is higher among such people. Chronic alcoholism, pellagra, sprue, tuberculosis, senility, malignant disease and cirrhosis among others, interfere with the proper nutrition of the patient and predispose towards vitamin deficiency.

The treatment of beriberi will depend upon the severity of the condition. There are no specific drugs of much value in treatment, although some of the orthodox cardiac drugs and the new diuretics (e.g., mersalyl) are sometimes used as adjuvants to vitamin therapy. In cases of severe or acute cardiac beriberi 20 mg. to 50 mg. of vitamin B₁ may be given daily parenterally, intravenously if need be [895]. Smaller doses repeated several times daily can be given instead. When the heart and circulation return to normal the dose can be reduced to 5 mg. to 10 mg. daily parenterally, and if there is no sign of gastro-intestinal upset this can finally be replaced by 5 mg. to 6 mg. daily by mouth. Hawes [244] describes the treatment of *shōshin* with injections of 5 mg. to 6 mg. of vitamin B₁ into the jugular vein, followed by 8 mg. at intervals of six hours. In

the chronic cases oral medication with 5 mg. to 6 mg. daily is usually adequate.

At the same time the diet, which should be a high protein, high calorie one (4,500 cal.), should be supplemented with whole grain cereals, yeast (40 gm. to 60 gm. daily), wheat germ and foods rich in vitamin B₁ and the vitamin B complex. Deficiency disease in man is never limited to a single factor, and a diet lacking in vitamin B₁ is lacking in the B complex, vitamins C and A, iron and minerals. Foods rich in these as well as liver and malt extracts should also be given. As much rest as possible should be taken, and complete rest in bed is indicated in severe cases with cardiac involvement. Venesection up to 8 to 10 oz. may be necessary if there is much distension of the right side of the heart, but in many cases the low blood pressure and anæmia do not permit this. Pleural effusions and ascites are relieved by paracentesis.

In addition to specific treatment the care of the limbs is important. In hospitalized cases a cradle is used to keep the bedclothes off the limbs, and a board for the feet to stop foot drop. The use of splints according to modern teaching is not recommended; if used they should be padded or made of plaster and bivalved. Gentle passive movements of all joints and paralysed limbs should be performed several times a day, and active movement encouraged as soon as possible, when splints are no longer needed. Hot fomentations in the early stage are probably useful. Massage and faradism are not employed until pain and tenderness have gone. For the relief of pain analgesics, such as aspirin or aspirin and codeine, are required, although heat in the form of baths and fomentations often relieves pain. Night cramps are treated with quinine 8 gr. to 6 gr. night and morning.

In the case of infantile beriberi intramuscular or intravenous injections of 5 to 10 mg. of vitamin B₁ in normal saline are given twice a day initially, and in the case of nursing mothers with affected children it is necessary to treat the mother as well. The infant should be taken from the breast and given cows' milk containing an added source of the vitamin B complex; tikitiki, an extract of rice polishings, is used in the East. When improvement sets in the dose of vitamin B₁ can be reduced or the same amount may be given orally.

Clinical response to vitamin B₁ therapy in acute beriberi is so dramatic that in acute heart cases improvement occurs in a few hours. The oedema, dyspnoea and tachycardia rapidly subside. Within a few days the patient may lose as much as five litres of oedema fluid [250] and in a fortnight the electrocardiogram may become normal and the heart smaller. In acute neuritic cases that are not far advanced relief from pain is experienced in a few days, and complete remission may occur in a few weeks. The chronic cases, however, in which the nerve degeneration has been progressive, may require a long time for recovery, and some residual neurological changes remain in cases of complete degeneration of the ganglion cells and axis cylinders. In the chronic neuritic cases the administration of foods rich in the vitamin B complex is essential.

OTHER MANIFESTATIONS OF VITAMIN B₁ DEFICIENCY

Owing to the ingestion of modern refined foods, including denatured cereals, it has been estimated that before the Second World War a third of the population of the British Isles existed on diets containing insufficient vitamin B₁, although at the moment the consumption of flour of eighty-five per cent. extraction has increased the average intake of vitamin B₁. It has been shown that disturbances to health may occur on diets deficient in vitamin B₁ long before the classical symptoms of beriberi are apparent. As far back as 1909 Fraser and Stanton [9] noticed that distinct physical impairment rapidly occurred on diets low in vitamin B, although a period of three to four months was needed for the appearance of frank beriberi. According to American writers peripheral neuropathy due to vitamin B₁ deficiency occurs in all economic classes, although it is more prevalent among the poor [481]. An examination of English medical literature would suggest that hypovitaminosis B₁ is less common in England, but this may be due to the fact that it passes undiagnosed.

Avitaminosis-B₁ may be complicated by vitamin C deficiency, so that in patients suffering from a combined deficiency, blood changes and hæmorrhages in the limbs and gums typical of scurvy may be superimposed upon the cardiac and neurological manifestations of beriberi. The condition is known as Rand scurvy or "ship beriberi," which may not only be found on the Rand and in ships, but amongst those living on unbalanced or poverty diets [678].

Factors conditioning Vitamin B₁ Deficiency. A state of vitamin deficiency, either mild or gross, may develop from a deficient intake due to a faulty diet, in which case the deficiency may be said to be primary, or it may result from factors other than an inadequate diet, that is from conditioned deficiency: A conditioned deficiency is caused by factors interfering with the ingestion, absorption or utilization of essential vitamins, or by factors that increase their requirement, destruction, or excretion [429]. The following table, taken from Jolliffe and Smith [429, 480], gives some of the factors conditioning vitamin deficiency:—

CONDITIONED VITAMIN DEFICIENCY

I. FACTORS INTERFERING WITH INGESTION

1. Personal :

Economic, ignorance, poor habits, food faddism, eccentricity, alcoholism, anxiety.

2. Gastro-intestinal disease :

Anorexia : due to alcohol, anæsthesia, post-operative, infectious disease and visceral pain.

Dysphagia.

Dyspepsia.

Nutritionally inadequate therapeutic diets as in :

(a) Gastro-enteritis.

(b) Cholecystitis and cholelithiasis.

(c) Ulcerative colitis.

(d) Peptic ulcer.

- (e) Obesity treatment.
 - (f) Chronic renal, hepatic and cardiac disease.
 - (g) Carcinoma of stomach and œsophagus, cardiospasm.
 - (h) Intestinal obstruction.
3. Food allergy.
 4. Mental disorders such as :
 - Neurasthenia.
 - Neurosis.
 - Psychoneurosis.
 - Psychosis.
 - Anorexia nervosa.
 - Migraine.
 5. Operations and anæsthesia.
 6. Loss of teeth.
 7. Heart failure (anorexia, nausea and vomiting due to visceral congestion).
 8. Parenteral administration of nutrients, *e.g.*, saline, glucose, amino-acids.
 9. Pulmonary disease (anorexia, vomiting due to cough).
 10. Toxæmia of pregnancy (nausea and vomiting).
 11. Neurological diseases interfering with self-feeding, chewing, and swallowing.

II. FACTORS INTERFERING WITH ABSORPTION

1. Diarrhœal diseases :
 - Ulcerative and mucous colitis.
 - Dysentery and intestinal parasites.
 - Intestinal tuberculosis.
 - Sprue.
2. Gastro-intestinal diseases associated with hypermotility or reduction of absorbing surfaces, *e.g.*, carcinoma, sprue, colitis.
3. Gastro-intestinal and external fistulæ.
4. Short-circuiting operations.
5. Vomiting.
6. Achlorhydria.
7. Biliary disease, especially obstructive jaundice.
8. Therapy—liquid paraffin, colloidal adsorbents and cathartics.

III. FACTORS INTERFERING WITH UTILIZATION

1. Hepatic dysfunction.
2. Diabetes.
3. Alcoholism.
4. Hypothyroidism.
5. Malignancy.
6. Therapy—sulphonamide drugs, radiation therapy, phenytoin.

IV. FACTORS INCREASING REQUIREMENT

1. Abnormal activity, *e.g.*, prolonged strenuous physical exertion.
 - Delirium.
 - Mania

2. Fever.
3. Hyperthyroidism.
4. Pregnancy and lactation.
5. Abnormal environmental factors :
Excessive temperature, as in tropics and certain industries.
6. Therapy increasing metabolic rate, such as thyroid, dinitrophenol, insulin, fever therapy, parenteral dextrose, high carbohydrate diets.

V. FACTORS CAUSING DESTRUCTION OF VITAMINS

1. Achlorhydria.
2. Lead poisoning ? Trinitrotoluene poisoning ?
3. Therapy with :
Alkalis.
Sulphonamides.
Arsenicals.

VI. FACTORS INCREASING EXCRETION

1. Polyuria as in :
Diabetes mellitus.
Diabetes insipidus.
Nephritis.
2. Lactation.
3. Excessive perspiration ?
4. Therapy : excessive fluid intake—urinary tract and other infections.

Not all these factors operate in the case of all vitamins. Thus the requirements of vitamin A are independent of carbohydrate intake, basal metabolism and temperature. These factors will be discussed in so far as they concern vitamin B₁.

I. Factors Interfering with Ingestion. An inadequate intake of vitamin B₁ may result from poverty, ignorance of what constitutes a balanced diet, poor food habits, food fads and eccentricity. We have seen patients with poor appetites, living on a high carbohydrate diet, who have neither the money nor the inclination to eat the right type of food, suffering from the manifestations of vitamin B deficiency—neurasthenic symptoms, fatigue, neuritis, sore tongue, mouth and lips, depression and insomnia [816]. Palmer [245] also describes a girl with eccentric food habits who lived largely on chocolate. Gastro-intestinal disease, especially if associated with anorexia, dysphagia, dyspepsia, pain or vomiting, such as occurs in nervous dyspepsia, peptic ulcer, gastro-enteritis, gall bladder disease or ulcerative colitis, are noted for their interference with food intake. The patient, either because of pain or nausea, limits the quality and quantity of his diet. The nervous tense woman with "nervous dyspepsia" or an "irritable colon" associated with abdominal pain who gradually reduces her diet to tea and toast, and who subsequently develops a sore tongue and signs of peripheral neuritis is also well known. Deficiency diseases of a mild degree occur not only among patients who have followed self-imposed diets for both real and imaginary complaints, but also among patients who have been dieted for long periods by their physician for such

conditions as allergy, peptic ulcer, biliary disease, nephritis, hypertension, colitis, diabetes and obesity. Wilbur [817] describes patients with marked loss of weight, stomatitis, peripheral neuritis and even pellagrous lesions following strict dietary treatment for non-organic digestive symptoms and for the relief of hay fever and asthma.

It is clear that many therapeutic diets even if well planned need to be supplemented by vitamins and minerals in which they are deficient. Particularly harmful are many of the slimming diets that are published in non-medical papers. Dried brewers' yeast and yeast extract are useful sources of the B vitamins for incorporation in therapeutic diets. Obstructive lesions such as carcinoma of the stomach and oesophagus and intestinal obstruction also limit food and hence vitamin intake.

In neuropsychiatric disorders, such as neurasthenia, the neuroses, psychoses, and anorexia nervosa the patient may have no desire for food. In migraine and hyperemesis gravidarum the mere sight of food may induce nausea. Anorexia is also associated with alcoholism, anæsthesia, post-operative convalescence, infectious disease and visceral pain, and if prolonged may lead to severe vitamin deprivation. Alcohol, which is poor in vitamins, produces deficiency disease by replacing other food and by causing nausea and vomiting by its irritant action on the stomach, in which it sets up a chronic gastritis. Alcoholism therefore produces vitamin B₁ deficiency by causing anorexia and by replacing vitamin B₁ containing foods. Civilized man usually satisfies anorexia not by a limited food intake all round, but by snacks of carbohydrate foods, such as bread, toast and sugared beverages, which increase the relative vitamin B₁ requirement. Anorexia may also occur in old people, particularly if edentulous and leading a solitary existence. Lack of teeth results in the consumption of pappy carbohydrate foods, which if refined are poor in vitamin B₁.

In heart failure and pulmonary disease the anorexia, nausea and vomiting due to visceral congestion and cough limit the intake of food. When saline, glucose and amino-acids are given parenterally because the patient cannot take food by mouth there is no vitamin B₁, except the small amount stored in the body, to metabolize the glucose. Vitamin B₁, nicotinic acid, riboflavin and vitamin C should be added to solutions for infusion if nothing is taken by mouth. Certain neurological diseases characterized by paralysis of the muscles of deglutition and cardiospasm may also interfere with the neuromuscular mechanism of swallowing. Patients with such conditions, as well as those with oesophageal stricture or carcinoma, or obstructive lesions in the gastro-intestinal tract, e.g., pyloric stenosis, carcinoma of the stomach, may fail to receive sufficient food by the oral route and suffer from deficiency disease.

II. Factors Interfering with Absorption. Diseases interfering with the absorption of food play an important part in producing conditioned deficiency disease, even if the intake of food is adequate in all respects. In diarrhoeal diseases, such as colitis (ulcerative and mucous), dysentery, intestinal parasitism [622], intestinal tuberculosis, sprue and pellagra, absorption of food and hence vitamins is impaired because it is rushed through the intestinal tract leaving little time for digestion, solution and

absorption [682, 430]. The absorbing surface of the gut may also be impaired, as in chronic ulcerative colitis and sprue, and the internal secretions may be so altered that absorption is imperfect. Absorption may also be modified by alkalis, adsorbents and lubricants (liquid paraffin) used in the treatment of these and other gastro-intestinal diseases. The water-soluble vitamins are adsorbed by such substances as aluminium hydroxide (*e.g.*, Aludrox) and kaolin, and liquid paraffin dissolves out the fat-soluble vitamins, A, D, E and K, which are thus lost to the body. Hydrochloric acid appears to be essential for the absorption of vitamins B₁ and C (p. 174), so that patients with achlorhydria may suffer from a deficiency of these vitamins. Because of its prevalence in elderly people, achlorhydria should be excluded in unexplained malnutrition before instituting treatment. Vomiting, which is a common symptom of gastro-intestinal disease, interferes with the absorption of food and if unrelieved may precipitate vitamin B₁ deficiency).

Obstructive gastro-intestinal lesions at or below the level of the stomach may interfere with the absorption of food. Thus a malfunctioning gastro-enteric anastomosis may obstruct the stomach and duodenum. Obstructive lesions of the small intestine have long been known to be associated with an anæmia and a clinical picture simulating that of pernicious anæmia and sprue.

Fistulæ, *e.g.*, gastro-colic, which short-circuit the small intestine, wholly or in part, are causes, albeit uncommon, of deficiency diseases. In spite of an adequate food intake so little may reach the small intestine that severe loss of weight and malnutrition may result. Syndromes resembling those of sprue and beriberi have been reported in patients with such fistulæ [218, 238]. Short-circuiting operations of the gastro-intestinal tract, *e.g.*, gastrectomy, have long been known to produce macrocytic anæmia and there is some evidence that other deficiency conditions may be produced. External intestinal fistulæ are uncommon, but they have been observed associated with deficiency disease.

Liver disease may lead to interference with the absorption of fat-soluble vitamins (A, D, E and K), and possibly with the intestinal absorption of vitamin B₁ [726].

III. Factors Interfering with Utilization. Evidence for the existence of factors interfering with the utilization of vitamins is largely circumstantial. It is known that certain vitamins cannot be utilized by the body as such. Thus carotene must be converted to vitamin A, vitamin B₁ to cocarboxylase, nicotinic acid to codehydrogenases I and II, and riboflavin to flavoprotein. The liver is considered to be the principal organ in which these conversions occur. The storage and utilization of vitamins A and K is impaired in liver dysfunction (pp. 17, 812).

Cirrhosis and other diseases of the liver are believed to inhibit the utilization of vitamin B₁, the excretion of which is lowered in hepatic disease. The high frequency of symptoms of vitamin B₁ deficiency in alcoholics is well known, and it is possible that hepatic dysfunction plays a part. It is believed by some that diabetes interferes with the utilization of vitamin B₁, although the evidence for this is conflicting. In malignant disease there appears to be a general vitamin deficiency.

Therapy with sulphonamides and other drugs may interfere with the utilization of the B vitamins. It is known that deficiency symptoms can be produced by administering sulphonamide drugs to animals, although this may be due to the inhibition of the intestinal synthesis of some of the B factors (pp. 120, 127). Sulphapyridine inhibits the activity of nicotinic acid in the experimental animal [664], but there is no evidence that it does in the pellagrin [429]. Radiation sickness has been attributed to failure of coenzyme formation from vitamin B₁ and nicotinic acid.

IV. Factors Increasing Requirement. It has already been stated that the vitamin B₁ requirement is proportional to the metabolic rate (p. 182). Fever increases the basal metabolism by 7·2 per cent. for each degree F., while strenuous physical exertion may increase it as much as fifteen times. The pyrexial patient therefore requires more vitamin B₁ than normal. The occurrence of vitamin B₁ deficiency conditioned by fever, hyperthyroidism (p. 190), pregnancy and lactation (p. 266) is well recognized. Johnson and his co-workers [818] have shown an increased requirement of the B vitamins produced by moderately strenuous physical activity in farmers, soldiers and other workers. On a consumption of 4,000 to 5,000 calories daily deterioration in physical fitness occurred on diets deficient in the vitamin B complex. This was remedied by giving the entire B complex. An increased requirement is therefore to be expected in occupations associated with prolonged strenuous physical work and in mental patients with delirium or mania, showing an increase in psychomotor activity. Outbreaks of deficiency diseases such as pellagra were once common in mental hospitals. Excessively high environmental temperatures, such as might be experienced in the tropics and in certain industries also increase the vitamin B₁ requirements (p. 191) and may bring on deficiency symptoms.

By increasing total metabolism various forms of therapy may produce a conditioned deficiency of vitamin B₁. Drugs such as thyroid, thyroxine, dinitrophenol, and fever therapy increase metabolism and hence the need for vitamin B₁. Parenteral administration of glucose, insulin therapy and high carbohydrate diets do not increase total metabolism, but increase the number of non-fat calories consumed and therefore the requirement of vitamin B₁. The long-continued administration of glucose to patients on a poor diet may precipitate deficiency disease [665, 667]. When glucose is administered in this way over a prolonged period vitamin B₁, nicotinic acid and riboflavin should be given as well. Excessive carbohydrate in the diet can also induce deficiency disease; beriberi due to this cause has, in fact, been reported [819].

V. Factors Causing Destruction of Vitamins. Melnick, Robinson and Field [820] have shown that vitamin B₁ is stable in gastric juice between a range of pH 1·5 to 8·0. The presence of antacids, bile or pancreatic juice destroys vitamin B₁. The administration of alkalis, as for peptic ulcer therapy diminishes the excretion of vitamin B₁. Achlorhydria also interferes with the absorption of the vitamin [820]. Recent work suggests that some of the vitamins are concerned with the detoxication of drugs such as sulphonamides and arsenical drugs (pp. 482-485).

VI. Factors Increasing Excretion. The possibility of the washing out

of the body of the water-soluble vitamins must be considered in conditions associated with polyuria, such as diabetes mellitus and diabetes insipidus. As Cowgill and his collaborators [224] have shown, excessive diuresis produced by forcing fluids can bring on the symptoms of vitamin B₁ deficiency. The forcing of fluids over a long period of time as a therapeutic measure in general infections and infections of the urinary tract, and the loss of fluid by diuresis in cedema may precipitate a deficiency. Lactation also results in a loss of vitamin B₁ from the body (p. 189), and is notorious in the East as a factor in the production of beriberi. Excessive perspiration has been said to result in appreciable losses of vitamin B₁ from the body, but this has not been confirmed (p. 179). In any case, loss of vitamin B₁ by this route would only be of significance under tropical conditions or in subjects doing heavy manual work in a hot environment. Certain drugs are known to increase the urinary excretion of vitamin C (p. 508), but it is not known to what extent the urinary excretion of vitamin B₁ is affected by drugs.

Biochemically Induced Vitamin B₁ Deficiency. It has been recognized that a compound may so structurally resemble a vitamin or growth factor that it will be adsorbed in an enzyme system containing the vitamin in place of the latter. The difference, however, is sufficient to prevent the cycle of chemical reactions occurring. The enzyme system is thus "blocked," e.g., sulphanilamide blocks the system containing *p*-aminobenzoic acid (p. 182) and pantooyltaurine blocks that containing pantothenic acid (p. 120). If the enzyme system of which the vitamin is a component is blocked, the latter is not available for growth and development of the organism. Pantothenic acid and *p*-aminobenzoic deficiencies can be produced in animals by feeding the appropriate inactivating agent. Woolley and White [905] have produced vitamin B₁ deficiency in mice by the addition of a pyridine analogue of thiamin known as pyrithiamin. If sufficiently large doses of vitamin B₁ are given to the animals recovery occurs. A competition between vitamin B₁ and pyrithiamin definitely occurs. Animals fed large amounts of vitamin B₁ and a small amount of pyrithiamin fail to develop deficiency symptoms, but do so if the amount of pyrithiamin is increased sufficiently. Experiments involving the feeding of a biochemical inhibitor may considerably simplify the study of vitamin deficiency disease, as they circumvent the use of synthetic or purified diets, which are laborious to prepare and unappetizing to eat. In human experiments particularly the consumption of an unpalatable diet may vitiate any results obtained.

Nervous Lesions in Vitamin B₁ Deficiency. In recent years there has been a tendency to ascribe many of the changes occurring in experimental vitamin B₁ deprivation to starvation rather than lack of the vitamin. Thus as far back as 1911 it was shown that fowls dying from starvation had peripheral nerve degeneration which could not be distinguished from that produced by vitamin B₁ deficiency [252]. Engel and Phillips [258] put forward a new and striking viewpoint concerning the relation of vitamin B₁ to nerve lesions. They administered β -carotene or percomorph oil and riboflavin to vitamin B₁ deficient chicks and reported that there was no sign of a pathological condition in the nervous system. They

therefore concluded that the nervous degeneration seen in human vitamin B₁ deficiency is due to lack of dietary factors other than vitamin B₁. They consider that the striking response to vitamin B₁ therapy is due to improved appetite and assimilation, and is not specifically related to nerve lesions. Their work is criticized by Cowgill [58]. Elvehjem [254] and his co-workers support the view of Engel and Phillips on chemical grounds and conclude that nutritional polyneuritis is the result of a general disturbance of carbohydrate metabolism [696, 698]. Certainly the functional activity of the central nervous system is dependent on respiratory metabolism, which is limited to the oxidation of carbohydrate. This is known, however, to require a number of specific catalysts of which vitamin B₁ and nicotinic acid are components. The recent work of Swank and Prados [782] has shown without doubt the causative connection between vitamin B₁ deficiency and the production of neurological lesions. They showed that vitamin B₁ deficient pigeons with ataxia and leg weakness and degeneration of cranial nerves recovered and were restored to a normal condition in a few weeks by supplements of vitamin B₁ given by tube; no other factor was given.

In animals suffering from acute vitamin B₁ deficiency Wallerian degeneration of peripheral nerve fibres occurs [289], and degenerative changes have also been described in the anterior and posterior nerve roots, tracts of the spinal cord, the medulla, pons, midbrain and internal capsule. In the deficient pigeon the first neuronal histological change is degeneration of the distal part of the axon, which proceeds towards the cell body, which becomes sclerosed; the large nerve fibres degenerate first [782]. If the deficiency is chronic degeneration of the vestibular nerves results, which is often associated with cerebellar ataxia, and sometimes degeneration of the cell bodies and peripheral fibres of the third and fourth cranial nerves. There is a progressive increase in the amplitude of the potentials, up to about three times normal, in the encephalogram of pigeons with vitamin B₁ deficiency. In the final stages there is a marked slowing down and depression of the brain potentials. The electroencephalogram returns to normal in a few hours if vitamin B₁ is given [821]. The neurological manifestations of vitamin B₁ deficiency in the pigeon are accompanied first by impaired function and then by degeneration of primary neurons of the proprioceptive nervous system and the central terminations of the optic nerves; after prolonged deficiency the efferent nervous system becomes affected. A number of degenerated fibres regenerate when vitamin B₁ is given, but severely injured ones continue to degenerate [782]. The vascular changes in the brains of vitamin B₁ deficient animals have been studied by Prados and Swank [882], who found that hæmorrhages occurred in the brain accompanied by perivascular sclerosis and interstitial cell proliferation. The hæmorrhages were preceded by vasodilatation and were first perivascular and later infiltrating. The lesions were accompanied or preceded by degenerative changes in the neighbouring neurons and swelling or hypertrophy of the oligodendrocytes and astrocytes. Everett [926] has studied the results of vitamin B₁ deficiency on the nervous system of the cat. After an initial period of anorexia the animal suffers from tonic convulsive seizures and disturbances of postural mechanisms,

such as impairment of labyrinthine righting reactions, the vestibulo-ocular reflex and the pupillary light reflex. Dysfunction of the cerebellum is suggested by asynergia, ataxia and dysmetria.

It has been suggested that the brain lesions are due to the accumulation of lactic and pyruvic acids, which when injected in small quantities (0.0002 to 0.0004 gm.) into pigeons' brains produce convulsions similar to those observed in pigeons deprived of vitamin B₁ [828]. This work might be of some significance if it had been controlled against other organic acids or even an inert fluid such as saline. The cardiac manifestations of vitamin B₁ deficiency cannot be produced by the injection of lactic or pyruvic acids [699]. If an animal is kept on a diet deficient in vitamin B₁ for a sufficient length of time, neurological changes that are irreversible are produced [822]. Thus dogs maintained for a year on a vitamin B₁ deficient diet show signs of irreversible peripheral neuropathy, and post-mortem exhibit involvement of peripheral nerves and anterior roots and posterior columns of the spinal cord, which persist after treatment.

In the human subject central degeneration is rare, although it may occur in beriberi [256]. The nerves supplying the lower extremities are usually affected, the outstanding symptoms being intermittent tenderness of the calf muscles, burning of the soles of the feet, skin hyperæsthesia in a sock distribution, anæsthesia, muscular weakness and cramps. Tenderness of the calf muscles and hyperæsthesia of the soles are the earliest symptoms. Such symptoms, which are bilateral, can be observed before the onset of typical neuritic beriberi, in which diminished reflexes and muscular paralysis and atrophy make their appearance. Vibration and position sense are impaired and ankle jerks may disappear. The terminal portions of the nerves are first affected and therefore the symptoms are more pronounced in the distal regions of the extremities, chiefly the lower, in which the sciatic nerve and its branches suffer. Histological changes are of late onset and may be still seen after symptomatic improvement. The first stages of recovery from the neuritis may be rapid, but many weeks may elapse before the complete use of the legs is regained. The slow improvement in severe neuritic cases under treatment is undoubtedly due to the long time taken for the remyelination of the nerve fibres. A unilateral peripheral neuritis which does not affect the legs first and foremost is not due to vitamin B₁ deficiency.

In moderately advanced cases pain along nerve trunks and along intercostal nerves may be complained of. Dropped foot and wrist, and muscular atrophy, especially of the thigh may result, and then the upper extremities may be affected. As the sensory changes advance, pain and numbness increase. Finally the muscular and sensory changes may involve the trunk, contractures may occur, and the patient becomes bedridden. Involvement of the vagus may produce paresis of the vocal cords. The oculomotor nerves, particularly the abducent, may be affected, as in Wernicke's encephalopathy (p. 216). A neuritis of the tenth nerve, or its laryngeal branches, leading to changes in the voice have been described [54]. Lesions of the optic nerve—diminished visual fields, temporal pallor of the disc, and primary optic atrophy—have been described in patients suffering from severe vitamin B deficiency, although several factors of the

vitamin B complex were probably involved [751]. Retrobulbar neuritis in pregnancy, diabetes and alcoholism have been attributed to vitamin B₁ deficiency [758].

The neurological symptoms that may occur in such diverse conditions as Korsakoff's syndrome [257, 258]; nutritional, gastrogenous [259, 260, 261], gestational [168, 262, 263, 264, 265] and "alcoholic" polyneuritis [266, 267, 268, 269]; achlorhydric hypochromic anæmia [275]; diabetes [270, 271], cancer [272] and tuberculosis [273] have been attributed to hypovitaminosis B. Sinclair [274] found that in a total of four hundred clinical cases the vitamin B₁ content of body fluids was below normal in pregnancy on a poor diet, macrocytic anæmia of pregnancy, pernicious anæmia with myxœdema, idiopathic steatorrhœa and coeliac disease, idiopathic hypochromic anæmia; nutritional, gastrogenous, gestational, and "alcoholic" polyneuritis; scurvy, carcinoma, and peptic ulcer, and after gastrectomy, fatal pulmonary tuberculosis and anorexia nervosa. Apparently normal values were found in toxic, infective and diabetic polyneuritis, which appear to have no direct relationship to vitamin B₁ deficiency. These observations correspond very closely with those of Rowlands and Wilkinson [152].

The term "gastrogenous polyneuritis" has been suggested for the clinical syndrome of peripheral neuritis associated with gastric dysfunction. While it has been recognized for many years that faulty functioning of the gastro-intestinal tract is often associated with morbid changes in the peripheral nerves it has only recently been realized that the cause of the polyneuritis is a conditioned hypovitaminosis B₁ [259-261]. Sinclair found low values for vitamin B₁ in the blood in some cases of idiopathic hypochromic anæmia, and he suggests that this is due to impaired absorption consequent on the achlorhydria, or to actual destruction of the vitamin in the stomach.

Again, the patient with pyloric stenosis and other gastric lesions is usually on a diet poor in vitamin B₁. Laurent and Sinclair [259] have shown that definite vitamin B₁ deficiency exists in such cases owing to the restricted diet, repeated vomiting and achlorhydria. In colitis [276, 283], sprue, chronic non-specific diarrhœa [276], and conditions with intestinal lesions the intake of vitamin B₁ is limited by diarrhœa. Prolonged febrile illnesses are frequently associated with a restricted diet and anorexia. The fever increases the metabolic rate and raises the vitamin B₁ requirements as well as interfering with the assimilation and utilization of vitamin B₁. Cirrhosis and other diseases of the liver, arteriosclerosis, and other conditions in which vital organs are damaged may inhibit the utilization of vitamin B₁, and give rise to deficiency symptoms such as polyneuritis.

Increased excitability was observed by Lewy [418] in the radial nerves of a group of pregnant women taking diets known to be deficient in vitamin B. The degree of peripheral nerve change, as indicated by chronaximetric examination, coincided with the severity of the clinical manifestations of deficiency. Improvement following vitamin B therapy was observed chronaximetrically at the same time that clinical improvement was recorded.

The polyneuritis associated with chronic alcoholism can be readily explained. Alcoholic addicts consume a notoriously irregular and inadequate diet, lacking in vitamins. The high calorie value of the alcohol increases the requirements for vitamin B₁, which is lacking in alcoholic liquors. Moreover, the gastritis caused by the alcohol causes anorexia and interferes with the absorption of vitamin B₁. The alcoholic buys liquor rather than food. A vicious circle is eventually set up. That the polyneuritis is not caused by the alcohol is shown by the clinical improvement after the administration of large doses of vitamin B₁ while the patient is still drinking large quantities of alcohol. It is possible that the oxidation of alcohol in the body is promoted by vitamin B₁ [825]. Similarly the pregnant woman who suffers from nausea restricts her diet to concentrated carbohydrate foods poor in vitamin B₁. Her repeated vomiting results in the loss of much of the small amounts of the vitamin that she does manage to ingest. Further, her vitamin B₁ requirement is considerably increased by her condition (p. 189).

Wechsler [277] first suggested that diabetic neuritis might be due to a vitamin deficiency. Vorhaus, Williams and Watermann [271], Sciclounoff and Broccard [280] and Lawrence and Oakley [108] claim that the administration of vitamin B₁ relieves the neurological symptoms of diabetes. These include paræsthesiæ, hyperæsthesia, anæsthesia, proprioceptive disturbances, sphincter impairment and ataxia. Needles [281], however, studied the vitamin B₁ metabolism in a group of diabetics suffering from peripheral neuritis and concluded that the diet contained an adequate supply of the vitamin, according to the requirements stated by Cowgill.

Peripheral neuritis occurs in the later stages of tuberculosis, and it has been suggested by Schiro, Aring and Spies [278] that this is due to a nutritional defect. Westenbrink and Goudsmit [282] examined fifty-one cases of tuberculosis and found that the excretion of vitamin B₁ was low in most of them; on the other hand, Najjar and Holt [415] state that the excretion in tuberculous patients is normal. Senility is frequently associated with defective nutrition, loss of teeth, anorexia, and arterial sclerosis. Peripheral neuritis is also common and similar clinically to that seen in beriberi.

Some cases of nutritional neuropathy are not due to vitamin B₁ deficiency, as shown by the work of Grande and Jiménez [824], who investigated cases of neuropathy during the Spanish Civil War. They found that lactic acid was metabolized normally, and therefore concluded that the neuropathies they observed were not ætiologically related to vitamin B₁. The diet in Spain consisted largely of bread, lentils, rice and soup and was not alarmingly low in vitamin B₁. Pellagra was common, but not beriberi. These neuropathies were not cured by vitamin B₁, nicotinic acid or vitamin A, but responded to treatment with yeast.

Campbell and Biggart [284] have examined cases of the disease known as superior hæmorrhagic polioencephalitis, or Wernicke's encephalopathy, which is characterized clinically by paralysis of the eye muscles, a reeling gait and drowsiness which usually terminates in fatal coma. The pathological changes are remarkable for their strict localization, hæmorrhage and glial proliferation of the corpora mamillaria, the hypothalamus,

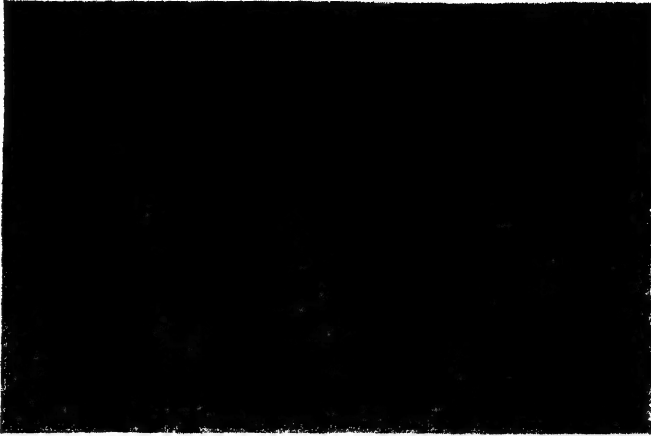


FIG. 41. Field from lesion in corpus mamillare, showing vascular dilatation and endothelial hyperplasia. Nissl stain. $\times 300$.



FIG. 42. Subacute case. Field from lesion in midbrain, showing vascular dilatation, thickening of capillary walls and cellular proliferation. $\times 65$.



FIG. 43. Field from lesion in corpus mamillare, showing fat (dark granules) in Sudan black B and hematoxylin. $\times 250$.

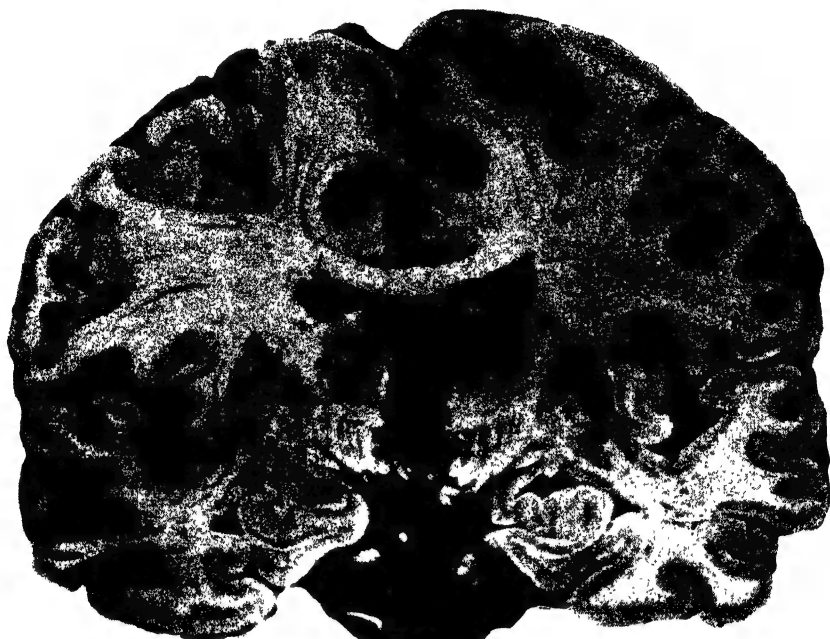


FIG. 44. Brain, coronal section, showing zone of congestion and petechiae around third ventricle and in corpora mamillaria.

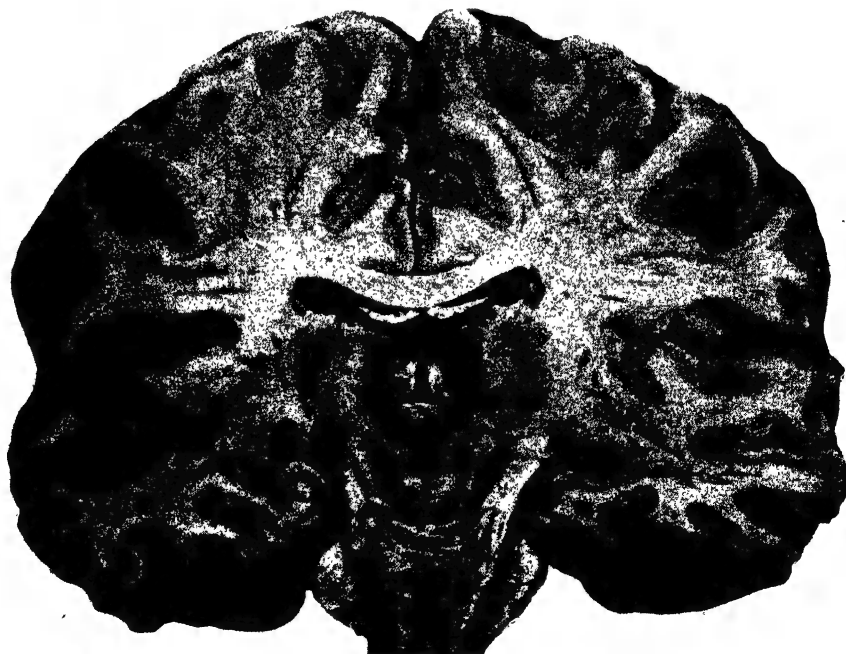


FIG. 45. Brain, coronal section, showing similar zonal lesion around posterior end of third ventricle and upper end of aqueduct.

the thalamus, the peri-aqueductal grey matter, and the colliculi and the floor of the fourth ventricle (Figs. 41 to 45). Wernicke's encephalopathy is closely related clinically to Korsakoff's syndrome, but its pathogenesis has always been in doubt. Of Campbell and Biggart's twelve cases only one showed signs of frank alcoholism, which in the past has been held responsible for the disease. The remainder occurred as complications of a wide range of primary conditions, the common factor of which was vitamin deficiency, especially of vitamin B₁. The disease occurs chiefly in alcoholics, but may also occur in diseases conditioning nutritional failure, *e.g.*, gastro-intestinal disease, tuberculosis, anorexia and hyperemesis gravidarum [785]. In a later paper based on the study of twenty-one cases, Campbell [710] considers that vitamin B₁ and nicotinic acid deficiencies may be factors in the causation of Wernicke's encephalopathy. Ecker and Woltman [545] have also presented evidence that B₁-avitaminosis occurred in a case observed by them. Experimental support for the view that the disease results from a vitamin B₁ deficiency was provided by Prickett [285], who produced foci of congestion, hæmorrhage and degeneration in the pons, medulla, and cerebellum of rats by feeding them on a diet deficient in vitamin B₁. Alexander [286] and his co-workers have produced Wernicke's encephalopathy in pigeons deficient in vitamin B₁ but receiving all the other vitamins. The cerebral lesions were identical with those seen in human cases. Wortis and Jolliffe [188, 743] have shown that the oculomotor palsies are due to vitamin B₁ deficiency and respond to treatment with the vitamin. The clouding of consciousness may be related to anything that interferes with brain metabolism such as lack of carbohydrate, oxygen lack, and lack of vitamin B₁, nicotinic acid and riboflavin. It is also possible that vitamin B₁ deficiency plays a prominent rôle in the ætiology of the alcoholic psychoses (p. 260).

Chastek paralysis in foxes, produced by feeding raw fish, is similar pathologically in the cerebral lesions to Wernicke's encephalopathy [828]. Raw fish contains an antivitamin which inactivates vitamin B₁ and produces the symptoms of vitamin B₁ deficiency in animals fed on it [829] (*cf.* avidin and inactivation of biotin, p. 125). The first symptom is anorexia, which is usually followed in a few days by weakness, hyperæsthesia, ataxia and death. The disease is definitely due to vitamin B₁ deprivation, as it may be produced experimentally and is curable by injecting vitamin B₁ [829]. Cooked fish has no such antivitamin B₁ action, nor has raw fish fed as a separate meal. The antivitamin B₁ factor, which is present mainly in the viscera, head, skin and scales, is destroyed on cooking or drying. Its action appears to be enzymatic. No epidemics resembling those reported in animals have been reported in man.

Psychological Manifestations of Vitamin B₁ Deficiency. A number of investigations by different groups of observers have been made during the last few years on induced vitamin B₁ deficiency in human volunteers. Certain mental symptoms, resembling those of neurasthenia have been reported by all investigators [827, 831, 697, 718, 715, 826]. These are described in detail on pp. 227-231. The principal manifestations are intolerance of noise, peculiar sensations in the head, inability to concentrate, inattention to details, memory defects, irritability, "nervous-

ness," anxiety, depression and insomnia. The most conspicuous are changes in attitude and behaviour and diminished inclination to perform accustomed work. The individual becomes depressed, unco-operative, apprehensive, irritable and quarrelsome, and partly from inability to concentrate on the work in hand and partly from inability to perform finer muscular movements minor accidents occur. These manifestations progress in intensity with prolonged deprivation of vitamin B₁ and are observed on intakes of 0.2 mg. or less per 1,000 calories.

Cardiovascular Lesions in Vitamin B₁ Deficiency. Human beings on diets deficient in vitamin B₁ may present cardiovascular disturbances, although they are not suffering from frank beriberi. Reports of cases of "beriberi" heart or heart failure due to vitamin B₁ deficiency are sufficiently scanty in England to justify the publication of one or two cases [288, 689-695, 827, 897, 907]. Tachycardia and arrhythmia are the most common disturbances of function, and are far commoner than the classical signs associated with severe deficiency, namely, peripheral neuritis and oedema. Other disturbances include peripheral vasodilatation, which produces a warm skin, right ventricular failure, low diastolic blood pressure and pistol shot sounds over the larger arteries [288].

Weiss and Wilkins [242], in America, studied a series of thirty-five cases suffering from nutritional deficiency with definite signs and symptoms of cardiovascular disease resembling the "beriberi heart" of previous workers. In addition they have found eighty-five similar cases in the Boston City Hospital. Bonsdorff [290] has also pointed out the rôle that nutritional deficiency may play in heart disease, and records that in 6,680 consecutive post mortems seven cases were found in which vitamin B₁ deficiency could be regarded as a cause of the heart lesions. The majority of Weiss and Wilkins' cases showed the following signs and symptoms: dyspnoea on exertion, reduced vital capacity, tachycardia, palpitation, gallop rhythm, cardiac and epigastric pulsation, a bounding peripheral pulse, distended veins and oedema. The skin was usually flushed and warm owing to capillary and arteriolar dilatation, and the velocity of the peripheral blood flow was increased. Roth, Williams and Sheard [927] were unable to confirm the vasomotor disturbances described by Weiss and Wilkins. Under controlled environmental, postural and metabolic conditions they failed to observe any changes in skin temperature, rates of cooling and warming of body tissues in subjects suffering from an induced deficiency of vitamin B₁ and other members of the vitamin B complex. Some degree of cardiac enlargement was present in most of Weiss and Wilkins' cases, but in some the heart was of normal size. The venous pressure was increased and nearly all cases showed some changes in the electrocardiogram, in which the chief alterations were an inverted T wave and a prolonged QT interval. Examination of the blood showed a rise in the blood sugar, and the bisulphite binding power of the blood (p. 286) and a lowered serum protein.

In studying this series Weiss and Wilkins placed the patients under observation on a vitamin B₁ deficient diet for from four to seven days, and then vitamin B₁ was administered intravenously or intramuscularly in doses of 20 to 50 mg. a day. This dosage was purely empirical and smaller



FIG. 46. X-ray of Patient suffering from Vitamin B₁ Deficiency, before and 20 days after Treatment. The measurements are in centimetres. The patient was an alcoholic who rarely ate more than one meal daily, and suffered from shortness of breath, tenderness of calves and legs and soles, pitting oedema of legs and an enlarged liver. The heart was enlarged on percussion and a systolic murmur was audible at the apex. The first X-ray shows enlargement of the heart, chiefly to the left. The second X-ray taken after 20 days treatment with 10 mg. vitamin B₁ daily shows return of heart shadow to normal size. The oedema and systolic murmur disappeared. (Drs. Porter and Downs case.)

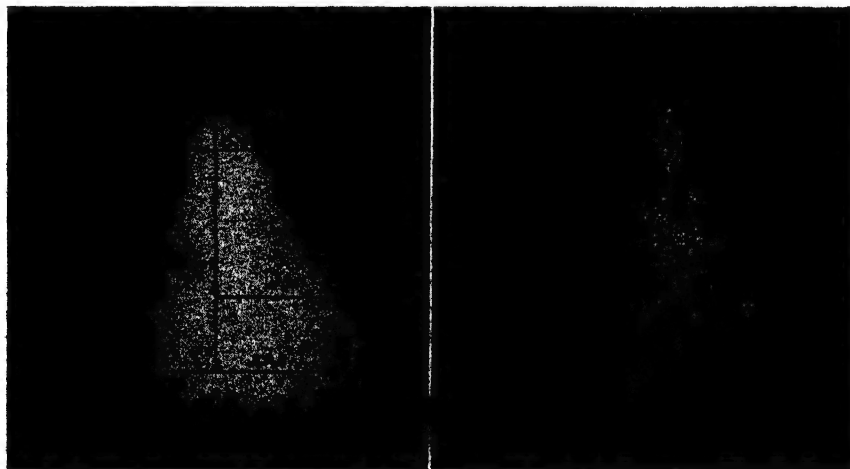


FIG. 47. X-ray of Patient suffering from Vitamin B₁ Deficiency, before and after 10 days after Treatment. Measurements in centimetres. The patient was an alcoholic taxi-driver living largely on snacks, who had swollen and painful legs, and tingling of the fingers. The condition became worse and the patient had an attack of delirium tremens with tremors, over-activity, hallucinations and disorientation. On examination the heart was enlarged, knee and ankle jerks were absent, muscle weakness and tenderness were present in the arms and legs, which showed pitting oedema. There was a high-pitched blowing systolic murmur, gallop rhythm and enlarged liver. The first X-ray shows enlargement of the heart to right and left. The second X-ray, showing return of the heart to normal size, was taken 10 days later, after daily injections of 12 mg. vitamin B₁ and yeast orally.

THE VITAMINS IN MEDICINE

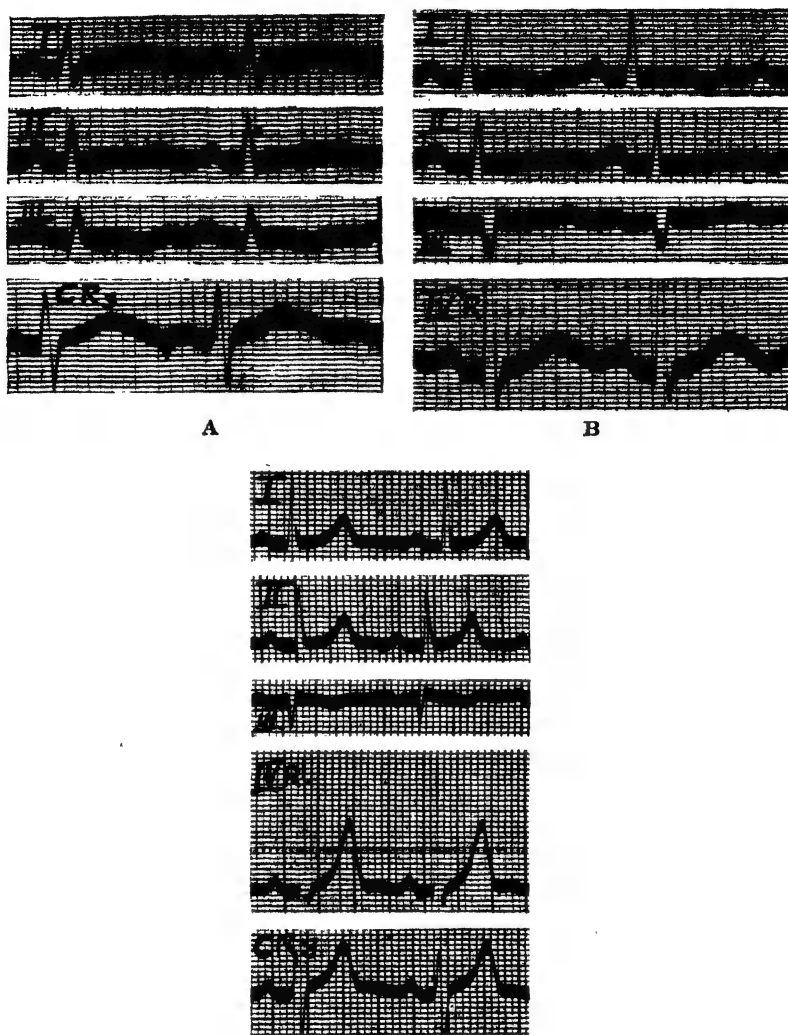
E.C.G. IN VITAMIN B₁ DEFICIENCY

FIG. 48. Electrocardiograms from a Case of Circulatory Failure due to Vitamin B₁ Deficiency. The patient was an alcoholic with a typical history—shortness of breath, oedema of extremities, systolic murmur over the apex, enlarged liver and heart. The first E.C.G. shows sinus tachycardia, main deflections upwards in all limb leads, small S₁ and Q₂, T₁ wide shallow and upright, T₂ almost absent and T₃ inverted, and QT interval greatly increased (0.49 sec.). The second E.C.G. was taken 20 days after treatment with vitamin B₁ (20 mg. daily by mouth and 7 injections of 25 mg.). R₁ has decreased in height, the main deflections are downwards, T₁ is inverted, T₂ is upright, and RT₁ is curved with the convexity upwards. The QT interval is still increased to 0.5 sec. The tracing is similar to those seen in coronary disease. The third E.C.G., taken ten months after the first, shows normal tracings for all leads, and normal duration of electrical systole (QT = 0.42 sec.). Time markings in A and B are 1/25 sec. and 1/25 and 1/5 sec. in C.

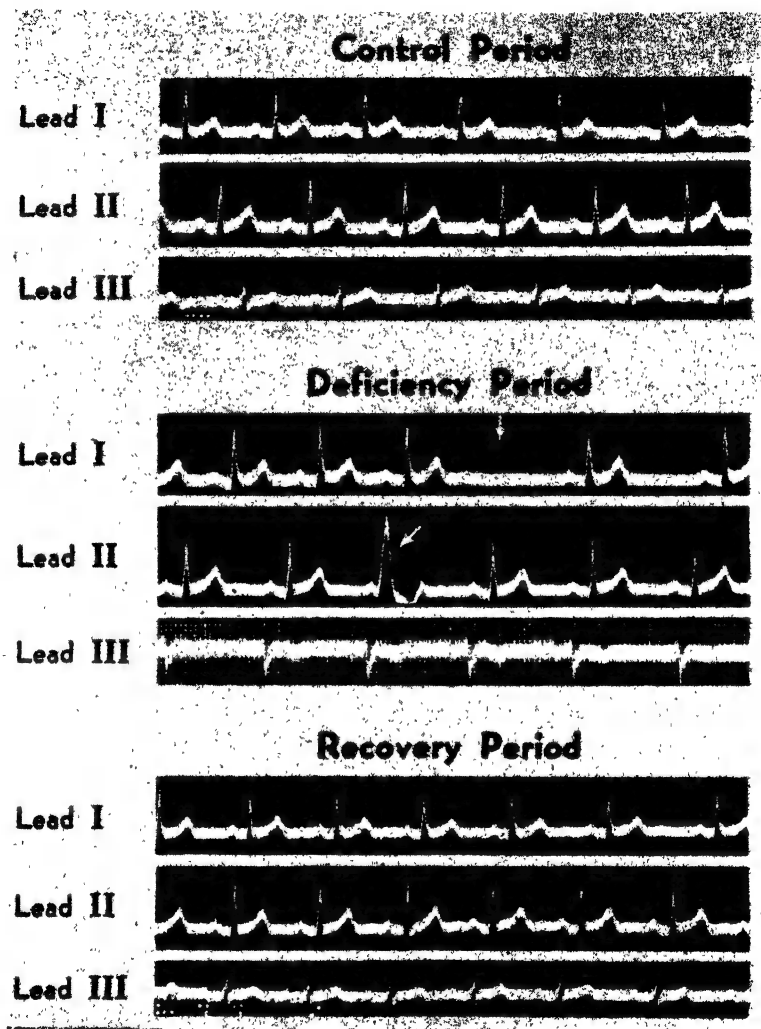
E.C.G. IN INDUCED VITAMIN B₁ DEFICIENCY

FIG. 49. Electrocardiograms in a Case of induced Vitamin B₁ Deficiency. The subject was placed on a diet of 0.47 mg. vitamin B₁ daily and a vitamin B₁/calorie ratio of 1. Changes in the E.C.G. occurred on the eleventh day after commencing the diet. They include sinus arrhythmia, sinus arrest, change in deviation of electrical axis and inversion of T_w. After administering adequate vitamin B₁ the E.C.G. was restored to normal on the fifth day of treatment.

doses would no doubt be just as effective. Remarkable improvement was often seen within a few hours, especially in patients with congestive heart failure. Five cases out of thirty-five died from acute heart failure, but complete recovery from cardiovascular manifestations occurred in the remainder. If the patient is deprived of vitamin B₁ for a sufficiently long period irreversible myocardial changes—such as hydropic swelling and vacuolation of the fibres—may occur [242, 882]. Repeated attacks of vitamin B₁ deficiency may leave a residuum of permanent damage in the heart. In the rat deprived of vitamin B₁ necrosis of muscle fibres, cellular infiltration and proliferation, and moderate fibrosis in the auricle have been described [896].

The frequency of this form of cardiovascular disease varies with the economic and social level of the population. Weiss [629] noted its occurrence in one of every hundred and sixty medical admissions in a large hospital. Since vitamin B₁ is only effective in cases of B₁-hypovitaminosis it is quite useless to administer it in heart disease or oedema due to any other cause. This has been proved clinically by Weiss [629].

The human electrocardiogram in vitamin B₁ deficiency shows changes, but they are not characteristic [242, 801, 827, 828, 696]. Common features that have been described are an increase in the length of systole, a rapid rate, a tendency to low voltage, a shortening of the PR interval, increase in the QT interval, sinus tachycardia, flattening of the T waves in leads I, II and III, and depressed ST segments. Williams and his co-workers [827, 696] state that on daily intakes of 0.1 mg. of vitamin B₁ for three months there are definite changes in the E.C.G. They describe a diminution in the amplitude of all complexes, particularly the T waves, which in lead IV in two cases became isoelectric, indefinite or shallowly inverted, and a prolonged QT interval. Jolliffe [828] also describes axis deviation and sinus arrhythmia. These changes in the electrocardiogram can be reversed after a few weeks by intensive treatment with vitamin B₁, *e.g.*, 10 mg. daily, unless the patient is suffering from chronic nutritional deficiency, in which case some of the cardiac lesions may be permanent.

The recognition of cardiac dysfunction resulting from B₁-hypovitaminosis is based on the following diagnostic criteria :

A history of nutritional deficiency, either chronic or acute ; fatigue and dyspnoea on exertion not otherwise accounted for ; tachycardia not due to hyperthyroidism ; palpitations ; dilated jugular veins ; arrhythmia ; right-sided enlargement of the heart (Figs. 46, 47) and increased circulation time ; electrocardiographic changes such as an indefinite T wave and prolongation of the QT interval, reversed by vitamin B₁ therapy (Figs. 48 and 49) ; oedema not in keeping with the cardiac picture, the state of renal sufficiency and the plasma protein level. According to Porter and Downs [881] there is an increased cardiac output, decreased arterio-venous oxygen difference, and an increased oxygen consumption.

Cardiovascular disturbances occur in a third of the subjects suffering from vitamin B₁ deficiency with neuritic symptoms. In the pig vitamin B₁ deficiency can cause cardiac dilatation and myocardial necrosis without any changes in the nervous system [880].

It has been suggested that the accumulation of metabolites, such as

pyruvic and lactic acids and methyl glyoxal, is a causal factor in the production of the cardiac manifestations of vitamin B₁ deficiency. Haynes and Weiss [699] have shown by injecting these metabolites into B₁ deficient animals that this view is incorrect. Their observations support the theory that the cardiac manifestations depend on a defect in metabolism, rather than on a toxic effect of circulating metabolites, as suggested in infantile beriberi (p. 198).

Cases of cardiovascular disturbances due to vitamin B₁ deficiency, and responding to vitamin B₁ therapy, have also been recorded by a number of workers [250, 292-294, 680]. Bickel [294] has drawn attention to the part played by vitamin B₁ deficiency in the genesis of cardiovascular disorders in alcoholics, pregnant women, hyperthyroidism, and diabetes. These disorders, Bickel explains, consist chiefly of tachycardia, cardiac irritability, dyspnoea of effort, enlargement of the heart, arterial hypotension, oedema, enlarged liver and occasionally acute asystole. They are accompanied by only slight anatomical lesions and may be considered, at least in their first phases, as purely disturbances of function. Such cases are often refractory to cardiotonics and diuretics, and only improve after the administration of large doses of vitamin B₁. It is important to recognize the presence of vitamin B₁ deficiency as early as possible as chronic cases (*e.g.*, alcoholics) occasionally prove resistant even to intensive vitamin therapy. In the treatment of most of his cases Bickel recommends a dosage of 50 mg. vitamin B₁ given parenterally four times a week, or 10 to 20 mg. a day. Since such patients are undoubtedly suffering from multiple deficiencies they should be given foods rich in the vitamin B complex, as well as liver and yeast extracts, iron and vitamin C.

Vitamin B₁ Deficiency and Gastro-Intestinal Symptoms. The chief gastro-intestinal symptoms of vitamin B₁ deficiency are anorexia and nausea, which promptly disappear on administering the vitamin. There is no method, however, of determining clinically which cases of anorexia and nausea are due to vitamin B₁ deficiency, unless other signs are present. The chronic alcoholic, the patient on a "faddy" or restricted diet, the pregnant woman suffering from hyperemesis, and the patient with "beriberi heart" all experience anorexia. The administration of vitamin B₁ frequently restores appetite in a dramatic fashion, even in a day or so, and the change is often more striking in those cases in which there are no definite gastro-intestinal symptoms, *e.g.*, in cases of cardiac insufficiency due to avitaminosis [242].

The earliest symptoms of inadequate intake of vitamin B₁ in infants are loss of appetite and restricted growth. Cowgill [802] noted that many marasmic babies have their interest in food and the urge to eat restored by the administration of vitamin B₁. Price [219] compares the appearances of infantile beriberi with those of the marasmic infant: vomiting and constipation, fretfulness, restlessness, pale appearance, pitiful cries, colic.

Many authorities have observed a loss in gastro-intestinal tone in animals and human beings deprived of vitamin B₁. The stomach is often distended, there is vomiting [58], constipation [827] and diminished peristalsis [803, 804], the secretion of gastric juice is impaired [805, 806,

807, 827], and hypo- or achlorhydria are sometimes present [808, 833, 877]. In much of this experimental work, however, there was a deficiency of other vitamins besides B_1 as simple avitaminoses are not encountered clinically. In view of these findings it has often been suggested that the frequency of constipation in civilized man is due to vitamin B_1 deficiency, but such a statement is extremely difficult to prove. Dick [539] noted a loss of intestinal tonus in B_1 deficient rats that was restored by feeding the vitamin. Careful radiological studies have shown that gastro-intestinal hypotonicity, dilatation and stasis follow a pure vitamin B_1 deficiency in the rat; these changes cannot be accounted for by the loss of weight that occurs [736]. Decreased intestinal motility, confirmed radiologically, has also been described in animals deficient in vitamin B_1 [737]. Williams [827] noted that constipation was a symptom in experimental vitamin B_1 deficiency in human beings. Joslin, Helms [809] and Macy [181] have observed that the administration of food supplements containing vitamin B_1 decreases the incidence of constipation, but this may well be due to the bran and fibre in the vitamin B_1 preparations, rather than to the vitamin B_1 itself. It is very doubtful if it can be proved that the vitamin has any effect on the constipation of the normal person not suffering from hypovitaminosis, although numerous claims have been made to the contrary. In the healthy rhesus monkey it has no laxative effect [833].

Elsom [810] found that experimental vitamin B deficiency in a hospitalized patient resulted in loss of gastro-intestinal tone and motility, which was relieved by yeast. Here again there is no proof that it was the vitamin B_1 of the yeast that was effective. Takai [811] noted that constipation was a common symptom in mild cases of infantile beriberi, and he observed that the constipation was relieved in thirteen out of sixteen cases after administering vitamin B_1 concentrates.

According to Eddy and Dalldorf [248] it is difficult to demonstrate any lesions in the intestinal plexus in B_1 deficient animals, although McCarri-son [812] claimed to have observed a degeneration in the cells of Auerbach's plexus. The most definite gastro-intestinal lesions reported in the experimental animal deprived of vitamin B_1 seem to be erosions and ulcers of the stomach [812, 813]. It has been stated that lack of vitamin B_1 may produce peptic ulcers in human beings, but there is little evidence in favour of this. On the other hand peptic ulcer may produce a conditioned B_1 -hypovitaminosis (p. 208). Hartung [815] treated sixty peptic ulcer patients on diets rich in vitamins for three years and obtained no better results than with the Sippy treatment. Eddy and Dalldorf [248] have seen pyloric thickening and hypertrophy post mortem in elderly subjects living on deficiency diets. They state that in many of these old people surprising clinical improvement sets in if vitamin B concentrates are given. Achalasia of the pylorus has been attributed to changes in Auerbach's and Meissner's plexus as a result of chronic hypovitaminosis- B_1 [816].

Etzel [817] has studied the geographical incidence of megacolon in relation to the amount of vitamin B_1 in the diet. He examined 626 cases of megacolon between 1920 and 1938 and observed that 618 came from districts in São Paulo, where there is much poverty and the diet is

mainly carbohydrate and poor in vitamin B₁. The condition was rare on the Atlantic coast where food was plentiful and salaries high. In a later paper he suggests that cardiospasm, megacolon, achalasia of the pylorus and mega-ureter are varied manifestations of a degeneration of the intramural autonomic nerves in the organs affected, produced by a chronic or intermittent deficiency of vitamin B₁ [788]. Etzel [928] and Moore [929] claim that vitamin B₁ in doses of 10 to 20 mg. every two or three days is a useful adjunct to other therapeutic methods in the treatment of achalasia of the cardia and cardiospasm. It is more likely that these conditions cause an induced deficiency of vitamin B₁ by diminishing intake of the vitamin (*cf.*, p. 208).

As a result of clinical studies on seventy-five patients Fitts [818] concluded that vitamin B₁ deficiency in modern diets may lead to definite changes in the motor and secretory mechanisms of the gastro-intestinal tract. The gastro-intestinal signs and symptoms listed by Fitts include gastric atony, spastic colon, and hypo- and achlorhydria. Patients with these symptoms showed improvement when given vitamin B₁. According to Simpson [819] the gastro-intestinal syndrome includes eructations, flatus, headache and loss of weight. Dysphagia has also been described as a symptom of vitamin B₁ deficiency [606].

Studies by Mackie and Pound [820] have revealed that in the majority of cases of chronic ulcerative colitis there is evidence of deficiency disease, and they suggest that a relative deficiency of vitamin B₁ might account for the abnormal intestinal function observed after barium meals were given. Others have observed a very low vitamin B₁ excretion in spastic colitis [686]. Here again it may be argued that defective intestinal absorption was a factor conditioning vitamin deficiency.

It is extremely difficult to evaluate many of these clinical studies on gastro-intestinal function and vitamin B₁, especially when yeast, wheat germ and vitamin B₁ concentrates were used for treatment, as these contain many other constituents in addition to vitamin B₁.

Experimental B₁ Deficiency in Man. Recently studies have been made by several investigators in America on induced vitamin B₁ deficiency in human beings. Williams and his collaborators [827] observed the signs and symptoms produced in four young women volunteers by a diet deficient in vitamin B₁, but adequate in every other respect. In another study observations were made on eleven volunteers. For twenty-one weeks volunteers were placed on a diet containing 0.15 mg. of vitamin B₁ daily. The ratio of the number of I.U. of vitamin B₁ to the number of non-fat calories in the diet never exceeded 0.085, which is much below the ratio 0.25 proposed by Williams and Spies as the minimum for the prevention of vitamin B₁ deficiency. Anorexia, fatigue, loss of weight, insufficiency or complete absence of free gastric juice, constipation and tenderness of the calf muscles were observed in all cases, but the onset of these was later than had been anticipated. The electrocardiographic changes are described on pp. 220-224. Blood sugar curves showed slight impairment of carbohydrate tolerance, but the blood bisulphite binding substances were not abnormal. No cardiac dilatation was observed on X-ray examination, nor was any oedema noted. A slight normocytic

anæmia developed in the subjects of the first study, but subsequently a considerable degree of hyperchromic macrocytic anæmia developed in five out of eleven cases. The anæmia was not relieved by any other vitamin, copper or iron and was therefore assumed to be the result of vitamin B₁ deficiency [715]. After subcutaneous injections of 1 mg. vitamin B₁ were given every third day, subjective improvement occurred within a few hours of the first injection. The improvement in the electrocardiographic changes after the second dose was very striking. Studies on the urinary excretion of vitamin B₁ were also made (see p. 178).

In a second study on experimental hypovitaminosis-B₁, Williams and his collaborators concluded that a climatological factor may influence the rate of development of vitamin B₁ deficiency. Physical activity also affected the rate of development of deficiency. They pointed out that the deficiency syndrome differs from that of beriberi in that œdema and cardiac dilatation are absent. They therefore question the accepted view that vitamin B₁ deficiency is responsible for the classic features of beriberi. The early stages of deficiency closely resemble those of neurasthenia; the later stages simulate anorexia nervosa.

Williams stresses the parallelism between vitamin B₁ deficiency and neurasthenia. The following table gives the symptoms of both conditions, those of vitamin B₁ being in italics.

GENERAL SYMPTOMS: *Fatigue*, often on slight exertion, *loss of weight* following *failure of appetite*.

LOCAL SYMPTOMS. Alimentary: *Capricious appetite, anorexia, indigestion, distension, eructation, nausea, vomiting, constipation or diarrhœa, mucous colitis.*

Circulatory: Cardiac discomfort, *tachycardia, palpitation, pseudo-anginal sensations, heart irregularity.*

Vasomotor: *Pallor, blushing, sweating, coldness, heat.*

Genito-urinary: Impotence, nocturnal emissions, genito-urinary paræsthesiæ, *dysmenorrhœa, dyspareunia, frequency of micturition, increased urinary output.*

Respiratory: *Frequent "colds," shortness of breath,* increased respiratory rate and shallow breathing.

Nervous System: *Peculiar sensations in the head* and every part of the body, feelings of swelling of the scalp, "*band around the head,*" bursting and stuffiness of the head, *headache especially occipital, peculiar and uncomfortable and painful sensation in the abdomen, rectum and breasts.* An almost universal complaint is *baekache.* Giddiness and dizziness are common and insomnia rarely absent. There may be *photophobia, muscæ volitantes, eye muscle fatigue, ear noises, intolerance of ordinary sounds.*

Mental Symptoms: *Inability to concentrate, uncertain memory, fear of insanity, awkwardness, self consciousness, irritability, depression, phobias, anxieties.*

Achlorhydria or hypochlorhydria are constant findings in both neurasthenia and vitamin B₁ deficiency, as well as delayed emptying time of the stomach and bowel, and diminished amplitude of the T waves in the

electrocardiogram. The following are also common to both conditions : Muscular atony, nausea, vomiting, roughness of the skin, faint heart sounds, lowered blood pressure, tachycardia, sinus arrhythmia on exertion, states of apathy, photophobic trends, difficulty of thought and memory, abdominal distention, burning of the soles of the feet, numbness of the legs, fatigue of the ocular muscles, tenderness of the calf muscles, and depressed tendon reflexes. A lowering of the basal metabolic rate from ten to thirty-five per cent. was observed in another study and intolerance to cold was a frequent complaint [715]. When the intake of vitamin B₁ was below 0.95 mg. a day fatigue, irritability and poor appetite, soreness of muscles and constipation were observed. When the intake was raised to 2 mg. a day a sense of well-being with unusual stamina and enterprise was experienced [827].

In a third study on induced vitamin B₁ deficiency Williams and Mason [715] observed changes of behaviour and other objective evidences of psychosensory and psychomotor disturbances. The subjects who were on diets yielding 0.4 to 0.45 mg. of vitamin B₁ daily, became depressed, irritable, quarrelsome and fearful. They became inefficient at their work because of generalized weakness, were inattentive to details of their work, were confused in thought, uncertain of memory and lacked manual dexterity. Six out of eleven were unable to carry on with their work. These objective signs and symptoms appeared after several weeks on a diet poor in vitamin B₁ and disappeared after the administration of the vitamin. Other subjective signs and symptoms complained of were headache, backache, dysmenorrhoea, muscle soreness, gastric discomfort after meals, sleeplessness, tenseness, intolerance to noise and increased sensitivity to painful stimuli. Hulse and her co-workers [945] noted a decrease sense of well-being in volunteers receiving diets containing 0.2 mg. of vitamin B₁ daily ; this occurred in from seven to eighteen days of commencing the test.

Williams and his co-workers [798] have made a recent study of the polyneuropathy that follows prolonged vitamin B₁ deficiency in man. Volunteers were kept on a diet providing only 0.1 mg. of vitamin B₁ per 1,000 calories for 120 days. The first objective evidence of abnormality was a decrease in the urinary excretion of vitamin B₁ after a test dose of 1 mg. Almost simultaneously the pyruvic acid level in the blood after the administration of glucose became elevated and the subjects became listless and complained of anorexia and easy fatigue. Listlessness progressed to apathy, anorexia to nausea and fatigue to prostration as time went on, with diminished responses to test doses of vitamin B₁ and with an elevation of blood pyruvic acid. Complaints of paræsthesia in the legs were made and later objective signs of disturbances in the nervous pathways appeared. After 110 days on the restricted diet the signs and symptoms of polyneuropathy made their appearance. These were defects in the cutaneous sensory pathways, diminution or disappearance of the tendon reflexes and paralysis of the muscles of the thighs and legs. It is interesting to note that in the earlier studies of Williams and his co-workers muscle paralysis and loss of tendon reflexes were not observed—possibly because the restriction of vitamin B₁ was too severe for the experiment to

be carried on for any length of time. After giving test doses of vitamin B₁ to the subjects appetite was improved and activity resumed for a short period. The outstanding subjective features of a pure vitamin B₁ deficiency in man would therefore appear to be anorexia, fatigue and polyneuropathy.

Jolliffe [828] and his co-workers carried out observations on five human volunteers, who lived on diets containing 0.86 mg. vitamin B₁ daily. Definite signs and symptoms of avitaminosis-B₁ were observed in four of the five subjects, and they appeared as early as the third day. The symptoms included constipation, anorexia, dyspnoea on slight exertion, precordial pain, palpitation, fatigue and lassitude, burning of the feet and muscle cramps. The precordial pain, aching in character, was precipitated by effort and persisted for two to three hours. The objective signs observed were skin hyperæsthesia to pin-prick in a sock distribution, changes in the electrocardiogram (see p. 220) and calf muscle tenderness. When vitamin B₁ alone was added to the diet all symptoms disappeared within three days and the objective signs within six days. Subjective symptoms did not appear unless the excretion fell below 100 μ g daily, although objective signs, such as changes in the electrocardiogram did not develop until the excretion fell below 80 μ g daily. The rapid production of both symptoms and objective signs is commented upon by Jolliffe. Since the vitamin B₁ intake was only forty-two to sixty-two per cent. of the minimum requirement it is suggested that there is a high prevalence of mild vitamin B₁ deficiency in the general population, and that vague ill-health characterized by fatigue and persistent anorexia may be a manifestation of a suboptimal intake of vitamin B₁.

Studies on the results of an experimental vitamin B complex deficiency in human beings have been made by Elsom [881, 718] and her co-workers, who found that both clinical manifestations and metabolic changes were of much later onset than in Jolliffe's cases of pure vitamin B₁ deficiency. They did not appear until after five weeks on the experimental diet and were only striking after two months. After five weeks a complaint was made of dyspnoea, palpitation, tachycardia and sharp precordial pain, but the electrocardiogram was normal. Mild anorexia was present after a week on the diet. Other gastro-intestinal symptoms were heartburn, a sense of epigastric fullness, abdominal distension and constipation. Towards the end of the experiment nausea and vomiting were frequent. Radiological examination showed an increased calibre of the jejunal loops. Within three days of beginning the diet diminution in the electrical irritability of the peripheral nerves and some disturbance of sensation were observed. A steady decrease in electrical irritability and exaggeration of the clinical signs of peripheral nerve involvement occurred as deficiency progressed. Mental symptoms which appeared during the fifth week of deficiency included irritability, nervousness, depression, loss of memory and inability to concentrate. Blood studies revealed a decrease in the number of red blood cells, increase in the mean cell volume and hæmoglobin, macrocytosis, polychromasia and poikilocytosis. A high blood sugar, a decreased response to insulin, but no change in the blood B.B.S. were observed late in the deficiency period. General manifesta-

tions that appeared were excessive fatigue, a pale flabby inelastic skin, œdema of the extremities (after five to seven days), and loss of weight.

The subsequent administration of vitamin B₁ relieved the cardiovascular and neurological manifestations, and in part the mental symptoms and the anorexia. The remaining gastro-intestinal symptoms, the anæmia, and œdema failed to respond to vitamin B₁ and only disappeared when a full diet with yeast was taken. The mental symptoms were only completely relieved when yeast was added to the diet. It is of interest to note that vitamin B₁ had no effect on the œdema, but it caused an almost immediate improvement in strength, general appearance and skin texture.

O'Shea and Elsom [760] state that foresight and judgment, as measured by performance and maze tests, are impaired in subjects receiving diets containing half the vitamin B₁ demanded by the Cowgill formula (p. 181). Improvement occurs if vitamin B₁ or the B complex is given.

Johnson and his colleagues [836] subjected ten men to hard physical work on diets deficient in part of the vitamin B complex. Fatigue, deterioration in physical fitness, muscle pains, poor appetite and constipation were complained of in about two weeks. Vitamin B₁ alone in doses of 2 mg. daily did not clear up these symptoms, but brewers' yeast did. Similar observations were made by Egaña and others [697] on subjects doing manual labour. They also noted loss of ambition and efficiency, deterioration of physical fitness for exhausting exercise and poor recuperation. The deficiency symptoms were relieved by brewers' yeast. No changes were observed in the blood pressure; oxygen consumption and carbon dioxide excretion; blood sugar lactate and pyruvate. Only one subject out of seven showed changes in the electrocardiogram.

Keys and his collaborators [980] were unable to confirm the observations of Johnson and his co-workers. They placed volunteers on diets deficient in vitamin B₁, riboflavin and nicotinic acid and failed to observe any changes in the capacity to do hard work or anaerobic work; in speed, co-ordination and muscle strength; in blood lactate and pyruvate; or in the psychological picture. Their subjects received a diet containing 0.16 mg. vitamin B₁, 0.15 mg. riboflavin and 1.8 mg. of nicotinic acid daily. No deficiency symptoms were produced after consuming this diet for a fortnight.

Barborka, Foltz and Ivy [890] have carried out exercise tests on trained subjects given diets deficient in the vitamin B complex. They showed that early mild states of malnutrition prevent maximal work efficiency. Diets containing 0.65 mg. of vitamin B₁ and 0.94 mg. of riboflavin, that is about a third of the allowance for men doing active work, produced subjective symptoms of easy fatigue, irritability, anorexia, and leg pains in subjects doing active physical work. These symptoms disappeared and work output returned to normal within a few days following the restoration of the vitamin B complex to the diet.

These observations on induced vitamin B₁ deficiency support the view that beriberi is not caused by a deficiency of vitamin B₁ alone, because in not one case of induced vitamin B₁ deficiency was typical beriberi produced. Deficiency diseases are never limited to lack of a single factor and if a diet is deficient in vitamin B₁ it is likely to be deficient in all the other B vitamins.

Diagnosis, Treatment and Prevention of Vitamin B₁ Deficiency. Many mild clinical cases of vitamin B₁ deficiency have escaped notice because clinicians wait for the appearance of full-blown beriberi or peripheral neuropathy before diagnosing it. In the case of a syndrome of subhealth resembling neurasthenia or chronic nervous exhaustion a deficiency of vitamin B₁ or of the vitamin B complex should be suspected. The diagnosis of vitamin B₁ deficiency depends on a careful dietary history and a complete examination of the nervous and cardiovascular symptoms. Special attention should be paid to such neurological manifestations as tenderness of the calf muscles on squeezing, hyperæsthesia, anæsthesia over the anterior surface of the tibia, muscle cramps and muscular weakness, which appear before the more serious neurological complications. Lost or diminished ankle and knee jerks and vibration sense may also be observed. Anorexia, fatigue, dyspnoea on exertion, loss of weight and irritability are also early symptoms. Factors predisposing to nutritional deficiency should be looked for (p. 206) particularly mechanical, medical or surgical causes interfering with intake, assimilation and absorption. The dietary history should be taken carefully—a laborious and painstaking process if done properly. Vitamin B₁ deficiency should be suspected in indigent and low income groups, in alcoholics, and in persons with poor food habits and food idiosyncrasies. It is also well to remember that parenteral fluids given as the sole means of nutrition to a person who cannot eat may precipitate deficiency disease (p. 211). Vitamin B₁ and other water-soluble vitamins should be added to parenteral fluids if nothing is being taken by mouth. A therapeutic test with vitamin B₁ should be performed in suspected cases, but in a case with advanced neurological manifestations it may take weeks for a satisfactory response to be obtained. Vitamin B₁ deficiency may also be diagnosed by the help of special tests on blood and urine (pp. 234–242).

With regard to the treatment of vitamin B₁ deficiency the dietary measures laid down for the treatment of beriberi (p. 204) should be followed. Since there is likely to be a deficiency in other food factors, especially the vitamin B complex, the diet should be supplemented daily with about 60 grams of brewer's yeast or other preparations rich in the B complex, or 80 grams of aqueous liver extract (or 8 c.c. by injection daily), 400–500 mg. ascorbic acid, and 50,000 I.U. vitamin A until improvement is shown. In severe cases 20 mg. or more of vitamin B₁ are given parenterally every day until the signs of avitaminosis begin to clear up [481]. Airing and Spies [416] recommend the administration of very large doses in the treatment of severe neurological conditions associated with vitamin B₁ deficiency. They give at least 50 to 100 mg. parenterally each day, in several doses of 10 to 20 mg. each. Doses of 100 mg. are also recommended by Dolger and his co-workers [718]. When the patient is saturated, which is recognized by an odour of burnt rubber in the urine or by urinalysis, the dosage can be reduced to 10 mg. a day orally, provided gastro-intestinal absorption is considered adequate. Later the dosage can be reduced to 5 mg. a day. Jolliffe [887] suggests the following scheme of treatment. For mild deficiency, 10 to 30 mg. parenterally daily; for moderate deficiency, 20 to 50 mg. parenterally daily; and for severe

deficiencies, 50 to 800 mg. parenterally daily. While no harm comes from such large doses it is doubtful if they are really necessary, if it is remembered that the bulk of a dose of vitamin B₁ given parenterally is excreted in three hours. It would appear to be better to give smaller doses more frequently. The importance of giving the whole vitamin B complex as well as vitamin B₁ in the treatment of so-called vitamin B₁ deficiency has been stressed by Sydenstricker [767], who has observed that intensive treatment with one factor of the vitamin B complex may precipitate symptoms of a deficiency of another. Thus massive doses of vitamin B₁ may produce the manifestations of riboflavin deficiency by causing increased excretion of riboflavin [768]. In actual practice a deficiency syndrome due to lack of a single vitamin is seldom or never seen, since no combination of foods, however poor, is lacking in one vitamin only. This is particularly true of the B vitamins which are associated together in foodstuffs. A diet lacking in vitamin B₁ is also likely to be lacking in riboflavin, nicotinic acid and other members of the vitamin B complex. As in the case of beriberi ancillary treatment may be necessary, *e.g.* diuretics for oedema if present, and cradles, heat, passive and active movements, and later massage for the affected limbs. Analgesics may be needed for the pain. Further details are given on p. 204 under the treatment of beriberi. Response to treatment is usually dramatic, except in chronic neuritic cases which require intensive treatment for weeks or even months.

The first signs of improvement looked for are slowing of the pulse, diminution of neuritic pain, paræsthesiæ, and calf tenderness, and a sense of well being. Recovery of motor and sensory perception are slower and do not occur within the period of the therapeutic test. If no improvement occurs after ten daily injections of 10 or 30 mg. vitamin B₁, according to the severity of the case, curative effects are not to be expected and another diagnosis should be sought, lest some more serious condition be overlooked. If the case is ascertained to be one of vitamin B₁ deficiency, the vitamin is administered daily until complete recovery has occurred. The rate of regeneration of nerves is about 1 mm. per day, so that months, or even a year or more, may be required for complete regeneration of the nerves of the leg. In paralysed cases irreversible changes in the central nervous system may occur, leading to permanent loss of function (*cf.* pp. 213, 214).

For the prevention of vitamin B₁ deficiency among the general population the following measures are suggested: (a) Whole wheat bread and whole grain cereals should be substituted for white bread and refined cereals. In America white bread is fortified with vitamin B₁ and other vitamins (p. 155). The objection to whole grain bread is largely one of custom. Even gastric ulcer patients can tolerate it if it is gradually introduced into the diet [838]. (b) Consumption of sugar and alcohol should be reduced, and where refined foods deficient in vitamin B₁ are eaten about 0.5 mg. of vitamin B₁ should be added for every 1,000 calories consumed. (c) Daily supplements of preparations or foods rich in the entire vitamin B complex.

To obtain a diet adequate in vitamin B₁ at least 1 pint of milk, one or

two eggs, two servings of fruit (raw if possible), two servings of vegetable, and whole grain bread or cereals should be consumed daily, and for a balanced diet butter should be added. Nuts are good sources of vitamin B₁. It is realized that in war time such ideal diets cannot be obtained. Therapeutic diets, taken over a long period, must be carefully scrutinized for their vitamin content, and if inadequate they should be supplemented as suggested above or by the addition of synthetic vitamin B₁ and the B complex. To prevent vitamin B₁ deficiency in persons in ill-health the factors producing vitamin B₁ deficiency should be borne in mind (pp. 206-212).

LABORATORY METHODS USED FOR THE DETECTION OF VITAMIN B₁ DEFICIENCY

Several methods have been devised for the estimation of vitamin B₁ in body fluids for detecting vitamin B₁ deficiency. Estimations have also been done on cocarboxylase and pyruvic acid, the blood level of which is stated to be raised in subjects on diets deficient in vitamin B₁.

Bradycardia Method [848-852, 879]. This method was the one in general use for estimating vitamin B₁ in body fluids before the advent of chemical methods. It depends upon the cure of bradycardia, produced by a diet deficient in vitamin B₁, by the vitamin B₁ in the substance under examination. Electrocardiograms are taken before the test, when the heart rate is 500-550 a minute, and again after feeding on a deficient diet, when it drops to 850 a minute. An electrocardiogram is taken again after feeding the test substance. The increase in heart rate and its duration are roughly proportional to the amount of vitamin B₁ in the substance. The method has been criticized as being non-specific [880, 881].

Phycomyces Test [855-860]. Originally devised by Schopfer [855, 882], this method in the hands of Meiklejohn [856], Rowlands, Wilkinson [857], Sinclair [858, 883, 884] and others [859, 889, 840, 909, 948] has been adapted for the estimation of vitamin B₁ in whole blood, blood plasma, urine and cerebrospinal fluid. The method is based on the fact that the extent of growth of the mould *Phycomyces blakesleeana* is proportional within certain limits to the concentration of vitamin B₁ in the medium. After inoculating flasks containing a known amount of vitamin B₁ and the test substance respectively with spores of the mould *Phycomyces blakesleeana*, the flasks are set aside in the dark at 20° C. for ten days, when the mycelia of the mould are removed, washed, and dried. The weights of the mycelia are approximately proportional to the amount of vitamin B₁ present, both as the free vitamin and as cocarboxylase.

According to Sinclair the true values for the total vitamin B₁ in the blood of healthy adults as estimated by the *Phycomyces* method range from 5.5 to 10.5 μ g per 100 c.c. with a mean of $7.4 \pm 1.4 \mu$ g. Values of 4.5 or less he considers to be abnormally low.

Thiochrome Method. When vitamin B₁ is oxidized in an alkaline solution by potassium ferricyanide it is converted quantitatively into thiochrome, which is fluorescent under ultraviolet light. The thiochrome can be estimated fluorometrically by comparison with a standard solution.

This method for estimating vitamin B₁ was devised by Jansen [385], and adapted by Westenbrink and Goudsmit [386, 387] for its estimation in urine. The vitamin B₁ in the fluid under estimation is adsorbed on synthetic zeolite [391, 705], frankonite or similar material and eluted. The vitamin B₁ in the eluate is oxidized by dilute potassium ferricyanide solution in the presence of caustic soda to thiochrome. After extraction of the thiochrome with isobutanol it is estimated fluorometrically either with the naked eye or by a fluorometer. Karrer and Kubli [389, 390] consider that the fluorescence is measured better by the naked eye. Vitamin B₁ can also be adsorbed on yeast, from which it can be liberated on boiling [392].

Various improvements have been made in the method [392-403, 841-849, 949], which normally estimates only free vitamin B₁ and not cocarboxylase, although it can be adapted for the estimation of both [397, 405, 406]. The method can also be adapted for the estimation of vitamin B₁ in blood [393, 404, 411, 849, 883]. If the thiochrome method is used for the estimation of vitamin B₁ in body fluids it is important that the subject should not take aspirin, salicylates, quinine or related drugs [843, 893], or nicotinic acid in doses of 300 mg. or more [756] just beforehand. For a simplified method of estimating vitamin B₁ in urine by the thiochrome method the papers of Harris and Wang [400, 848] should be consulted. A micro-method based on the thiochrome technique has been devised by Hinton [849].

Diazo Methods. A method of estimating vitamin B₁ depending upon the formation of a coloured azo dye was proposed by Prebluda and McCollum [366, 376] and developed by Melnick and Field [368, 369], and Emmett and his co-workers [418]. The vitamin B₁ is extracted with butanol, adsorbed on permutit, eluted at pH2 with potassium chloride solution and the eluate is treated with alcohol and phenol, made neutral to thymol blue, and treated with diazotized *p*-aminoacetophenone. After standing overnight the solution is extracted with xylene and the colour of the extract compared with that of a similarly prepared standard. The method is specific for vitamin B₁ and does not estimate cocarboxylase. The Prebluda and McCollum method has been applied to the determination of both free and total vitamin B₁ in animal tissues and fluids, grains and fæces [850].

A method of estimating vitamin B₁ has also been devised depending on the formation of a red coloration when the vitamin reacts with the diazotized trichloracetate of ethyl *p*-aminobenzoate [848], and diazotized *p*-aminoacetophenone [884].

Estimation of Cocarboxylase or Vitamin B₁ Pyrophosphate [372-377, 406-408, 417, 714]. Since ninety per cent. of the vitamin B₁ present in the blood is in the form of the pyrophosphate or cocarboxylase, the estimation of the latter gives an indication of the degree of vitamin B₁ deficiency. An aqueous extract of yeast powder [407], diastase [874], or kidney [408] converts blood cocarboxylase quantitatively into vitamin B₁, which may then be estimated by conversion to thiochrome or by the diazo method. Goodhart and Sinclair [377] estimate blood cocarboxylase by measuring the volume of carbon dioxide liberated from pyruvic acid.

They have shown that the amount of cocarboxylase in blood varies directly with the amount of total vitamin B₁ and also with the degree of saturation of the tissues with the vitamin. In normal males on an unrestricted diet the blood cocarboxylase ranged from 4.5 to 12 μ g per 100 c.c., with a mean of 7.0 (\pm 2.1). Goodhart [417], who has modified the method by using washed blood cells, gives the mean value as 7.0 (\pm 1.52). Recently a micro-method for estimating small amounts of cocarboxylase (0.00005 μ g) has been devised by Westenbrink [425], who gives the cocarboxylase content of the blood as 12.2 \pm 1.5 μ g per 100 c.c. [851]. Schlutz and Knott [714] conclude that an infant or healthy young child has a blood-cocarboxylase level of 10 μ g or more per 100 c.c. Figures for children suffering from prolonged chronic or acute illnesses were much lower. Wortis [86] puts the normal figure for children between 6.5 μ g and 8 μ g.

Estimation of Pyruvic Acid and Bisulphite Binding Substances. It has been stated (p. 166) that an increase in pyruvic acid and bisulphite binding substances (B.B.S.) occurs in the blood and urine in vitamin B₁ deficiency. Many workers believe that the estimation of B.B.S. and pyruvic acid in the blood and urine may be used as an index of vitamin B₁ nutrition. Wortis and his co-workers [86], Melnick and Field [201] state that other substances besides pyruvic acid bind bisulphite and that elevation of B.B.S. is not a specific indication of vitamin B₁ deficiency. They claim to have observed an elevated B.B.S. value with a normal vitamin B₁ excretion, and a normal B.B.S. value in established vitamin B₁ deficiency. According to a number of other workers [84, 87, 88, 154, 314, 708] the amount of blood pyruvate runs parallel with the vitamin B₁ reserves of the body, and has been used as a measure of vitamin B₁ deficiency. Wortis and his co-workers [86, 719], while not accepting the level of B.B.S. in blood and cerebrospinal fluid as an index of vitamin B₁ nutrition, have, however, observed high B.B.S. levels in the blood of patients with peripheral neuropathy due to alcoholism, which is often associated with vitamin B₁ deficiency, in pyrexial patients, and after the ingestion of glucose by normal individuals. Klein and Elsom [899] found a normal blood pyruvic acid in a group of subjects receiving only half the minimal allowance of vitamin B₁. Some showed signs of subclinical deficiency.

Wilson [720], using the blood pyruvic acid as a criterion, was unable to obtain evidence of vitamin B₁ deficiency in English children of the hospital class. Patients with gastro-enteritis, nephritis and convulsions showed low levels.

According to Wortis and co-workers [719] the normal range of blood pyruvic acid in American subjects is 0.77 to 1.28 mg. per cent., with an average of 1.02 mg. Golberg and Gillman [852] give the figure 0.79 \pm 0.19 mg. for South African males and 0.85 \pm 0.19 mg. for females. Wortis gives the upper value of normal as 1.8 mg.

Williams and his colleagues [715, 793, 795] determined the concentration of B.B.S., pyruvic acid, lactic acid and glucose both after exercise and after the administration of glucose orally or intravenously, and they found elevated values for these in the more severe states of vitamin B₁ deficiency. They also noted raised values in insulin hypoglycæmia, hyperthyroidism and mild diabetes. If these factors and others related to

pyruvate metabolism are excluded Williams believes that a high blood pyruvic acid after the administration of glucose is significant of vitamin B₁ deficiency. For the intravenous test 0.4 gm. of glucose (0.8 c.c. of fifty per cent. solution) per kilogram of body weight is injected in three minutes. A sample of blood is taken just before and thirty, sixty, and one-hundred and twenty minutes after the injection. The highest value is obtained after thirty minutes and this is considered the most significant. For the oral test 50 gm. of glucose in 250 c.c. of solution are administered, and thirty minutes later a second dose of 50 gm. is given. Specimens of blood for pyruvic acid estimation are taken just before the first dose and thirty, sixty and ninety minutes later. The maximal values are obtained between sixty and ninety minutes. In severe vitamin B₁ deficiency, e.g., on an intake of 0.1 mg. per 1,000 calories, the blood pyruvic acid rises half an hour after the injection of glucose from 1.2 to 1.5 mg. per cent. to 2.8 to 2.6 mg. A return of blood pyruvic acid to normal after the administration of vitamin B₁ confirms the biochemical defect caused by vitamin B₁ deficiency. Blood pyruvic acid is also disturbed in poisoning by arsenic and phosphorus, in the pernicious vomiting of pregnancy, eclampsia and acute yellow atrophy of the liver. Hulse and her co-workers [945] confirm the observation that in subjects deficient in vitamin B₁ there is a delay in the return of the blood pyruvate concentration to normal after exercise.

Chesler, Homburger and Himwich [919] stress the fall in the lactic acid pyruvic acid ratio in vitamin B₁ deficiency. This is normally 7.7 and may fall to 5.8 to 6.6.

Yeast Fermentation Method. This method is based on the observation that vitamin B₁ increases the rate of production of carbon dioxide by yeast [362-365]. Various improvements, including a micro-method have been devised, so that estimations can be done on body fluids [526, 852]. Carleen and co-workers [908] have shown that the yeast stimulating activity of plasma is a measure of its vitamin B₁ content.

Bacterial Growth Method. A micro-method for estimating vitamin B₁ has recently been devised depending on the growth response of the organisms *Staphylococcus aureus* [361], *Streptococcus salivarius* [853] and *Lactobacillus fermentum* [946] to the vitamin.

Pyrimidine Estimation. It is claimed that the level of vitamin B₁ nutrition can be measured by estimating the increased excretion of pyrimidine, which is split off the vitamin B₁ molecule [724].

A comparison has been made between the bioassay and thiochrome methods. The latter gives values of the order of eighty to ninety per cent. of bioassay methods [854].

Estimation of Vitamin B₁ in Blood, Urine and Muscle. The various values obtained for the level of vitamin B₁ in the blood of normal individuals are given below. The values vary considerably, but average about 7 μ g per 100 c.c. According to Sinclair [858] ninety per cent. exists in the phosphorylated form as cocarboxylase. Sinclair [409] has also estimated vitamin B₁ in cerebrospinal fluid, but the range in health and disease is so wide (0.0 μ g to 6.5 μ g) that the estimation has no practical value in detecting vitamin B₁ deficiency. This has been confirmed [428]. Values

Vitamin B₁ Content of Blood

Investigator and Method.	Vitamin B ₁ in Blood in micrograms per 100 c.c.	Suggested Deficiency Level in micrograms per 100 c.c.
<i>Phycomyces</i>		
Meiklejohn [356]	6.5-14.0	—
Laurent and Sinclair [259]	—	7 or less
Sinclair [858]	5.5-10.5	—
	(mean 7.4 ± 1.4)	4.5 or less
Rowlands and Wilkinson [857]	6.5-16.5	5.5 or less
Lehman and Nielsen [410]	7-13	—
Guhr [360]	8-10	—
Deutsch [889]	9-16	—
	(3-7 in plasma)	
<i>Thiochrome</i>		
Schneider and Burger [411]	3.8-10	5.3 or less
	(mean 6.4)	
Widenbauer <i>et al.</i> [412]	2-11	4 or less
	(mean 7)	
Widenbauer [856].	10	—
Ritsert [393]	3-15	—
	(mean 7.9 ± 3.9)	
Hennessey and Cerecedo [408].	9-12	—
Magyar [624]	1-15	—
	(mean 7.6)	
Molnar and Morani [855]	19.5 ± 3.5	—
De Jong [857]	3-7	—
Benson <i>et al.</i> [862]	4.8-12.3	—
	(mean 7.8 ± 1.8)	
Williams <i>et al.</i> [296]	6-9	—
<i>Coccarboxylase</i>		
Goodhart and Sinclair [875, 877, 417,	4.5-12	3 or less
	(mean 7.0 ± 2.1)	(as coccarboxylase)
Westenbrink <i>et al.</i> [858]	9.0-13.5	—
Pannekoek and van Veen [688]	—	5.5 or less
<i>Fermentation</i>		
Goodhart and Nitzberg [859]	3.1-9.2	3.5 or less

less than $3\mu\text{g}$ to $7\mu\text{g}$ of vitamin B₁ per 100 c.c. of blood have been taken to indicate vitamin B₁ deficiency. The wide variation in the blood of normal persons suggests that the determination of blood vitamin B₁ is useless for the detection of vitamin B₁ deficiency. Thus Benson and co-workers [788] observed a range of $4.8\mu\text{g}$ to $12.3\mu\text{g}$ in one-hundred and twenty-one children, and twenty-two with unequivocal evidence of vitamin B₁ deficiency had blood levels within the normal range. Goodhart and Nitzberg [859] have reported that most patients with peripheral neuritis have vitamin B₁ levels of $3.5\mu\text{g}$ or less, but peripheral neuritis is not always nutritional in origin. It is reasonable to assume that the blood vitamin B₁ falls in cases of severe vitamin B₁ deficiency, when the stores of the vitamin in the tissues are almost exhausted. Then the urinary excretion of vitamin B₁ will be low. Random estimations of vitamin B₁ in blood and urine are valueless, as in most cases the values obtained reflect only the immediate intake of the vitamin.

The determination of vitamin B₁ in the urine has also been used as a means of estimating the degree of vitamin B₁ deficiency. According to Melnick [798] the body excretes forty to fifty per cent. of the vitamin B₁ in the diet if this is adequate; Benson [862] gives the figure twenty per

cent. The following table gives the daily urinary excretion of vitamin B₁ in normal persons and the suggested urinary excretion below which vitamin B₁ deficiency is thought to occur.

Vitamin B₁ Content of Urine

Investigator.	Method.	Micrograms of Vitamin B ₁ excreted in Urine in 24 hours in Persons receiving adequate Vitamin B ₁ .	Minimum Daily Excretion of Vitamin B ₁ in Micrograms, below which Deficiency is said to occur.
Harris and Leong [155] . . .	Bradycardia	72-105	30
Harris, Leong and Ungley [156]	"	36-105	30
		Average 66.	
Wright and Baker [158] . . .	"	120-480	39
		Mean 200.	
Melnick and Field [165, 369] . .	Diazo	128-850	90
Melnick and Field [861] . . .	"	400	Less than 60
Yang and Platt [370] . . .	"	20-125	
Jolliffe <i>et al.</i> [328] . . .	Thiochrome	300	100
Karrer and Kubli [389] . . .	"	97	—
Wang and Harris [157] . . .	"	150-240	90
Wang and Yudkin [167] . . .	"	137-233	66-89
Hills [388] . . .	"	50-170	—
		Mean 100.	
Westenbrink and Goudsmit . .	"	120-830	40
[886, 387].		Mean 230	
Ritsert [392] . . .	"	110-520	
Borson [414] . . .	"	100-300	
van Coeverden [168] . . .	"	100 or more	50 or less
Benson <i>et al</i> [862] . . .	"	150	—

Carleen and co-workers [860, 902, 903] have suggested another method for estimating the vitamin B₁ reserves of the body. They remove a small amount of muscle (5-15 mg.) with a sterile needle from the glutæus maximus, which is previously anaesthetized with procaine. The vitamin B₁ in the tissue is then estimated. Once standards can be laid down this method, although perhaps not comfortable for the patient, appears to be one of the best for determining the tissue reserves of the vitamin. The same workers have shown that the yeast stimulating activity of plasma is proportional to its vitamin B₁ content [908]. They propose to use the yeast stimulating activity of plasma and muscle as an index of vitamin B₁ nutrition [945].

Test Dose. The range of values is so great that unless the excretion of vitamin B₁ is exceedingly low it is of little value as an index of vitamin B₁ nutrition. The administration of a test dose would appear to give more information. If the tissue stores of vitamin B₁ are low and the vitamin is given by mouth or by injection, the tissues will take it up and very little will be excreted in the urine. On the other hand, if the tissue stores are adequate a considerable proportion of the test dose is excreted. There is considerable difference of opinion as to how much vitamin B₁ to give as a test dose, by what route it should be given, and how much of it is excreted by the individual receiving an adequate intake of vitamin B₁ in his food.

Another difficulty that is not always appreciated is that vitamin B₁ is not a threshold substance. The rate and amount of excretion depend on

the size of the test dose and the route of administration, and is not determined solely by the patient's nutritional status. A number of variable factors affect the rate at which the vitamin is absorbed and phosphorylated. Another pertinent fact pointed out by Ferrebee [785] is that the tissue concentrations at which symptoms of deficiency develop are probably different in different tissues, and probably vary with the amount of physiological activity at the time; this will in turn be reflected in the urinary excretion. A bedridden patient may show considerable loss of vitamin B₁ from the tissues before deficiency symptoms appear.

The effect of test doses on vitamin B₁ excretion was first examined by Harris and Leong [155], according to whom the extent of vitamin B₁ deficiency can be determined by finding the number of repeated test doses of 1 mg. which have to be given before the subject reaches a state of stable saturation at a normal level of excretion. The "resting level" of excretion is determined and 1 mg. of vitamin B₁ given daily until there is a response. In a person on a diet adequate in vitamin B₁ there is an immediate response to the first test dose, the increased excretion remaining approximately constant on each day that a test dose is continued and amounting to about twenty-five per cent. of the extra vitamin given. With the subject deficient in vitamin B₁ the plateau of excretion is not reached until at least the second day after giving the test dose. The output of vitamin B₁ falls to the original level on ceasing to give test doses [157].

Hills [888, 709] administers a test dose of 1 mg. vitamin B₁ by mouth and examines the excretion of the vitamin during the following three hours. The maximum response to the test dose occurs during the second hour. In normal subjects this ranged from 26 to 110 μ g, with a mean of 65 μ g. In subjects deficient in vitamin B₁ there is no response to a test dose or only a slight response. Saturation is reached when 400–600 μ g of the vitamin is excreted daily. The corresponding excretion for three hours after a 1 mg. test dose is 200 μ g. Melnick, Field and Robinson [165] consider that a daily excretion of 90 μ g and 60 μ g are the normal minimum values for men and women respectively, and that values below this should suggest evidence of a possible vitamin B₁ deficiency. They give a test dose of 5 mg. of vitamin B₁ with a meal. If ten to twenty per cent. of this dose is excreted there is no vitamin B₁ deficiency; if seven to ten per cent. a mild deficiency; and if less than seven per cent. a marked deficiency. The dose is given with a meal because if given on an empty stomach the vitamin B₁ is destroyed to some extent before absorption. Jowett [705] states that the shortest period for collecting the urine after a 5 mg. test dose is five hours. Although they prefer the oral route, Melnick and Field [782] state that if the test dose of 5 mg. is given parenterally the major excretion of the vitamin occurs in the next four hours. This is more convenient, and since it does not involve the collection of a number of urine specimens is suitable for use in out-patient clinics. The parenteral route also eliminates any variation in the rate and degree of intestinal absorption. An excretion of 50 μ g or more during the four hours after test dosing was considered evidence of adequate vitamin B₁ nutrition. Melnick and Field also followed (a) the basal twenty-four hours urinary excretion of vitamin

B₁, (b) the fasting four-hour excretion, (c) the four-hour excretion after the administration of 350 μ g of vitamin B₁ per square metre of body area (approximately 0.65 mg. for the average male). These gave good agreement with the oral and parenteral test dose methods.

A standard test dose of 0.1 mg. per kilogram of body weight is suggested by Borson [414], who states that eight to ten per cent. is excreted in a normal person, most of it within the first five hours. The extent of deficiency is calculated by determining the number of test doses that must be administered for excretion to rise to the normal level, which in subjects receiving a diet adequate in vitamin B₁ is 100 to 300 μ g daily according to Borson. In patients with severe vitamin B₁ deficiency as much as 100 mg. was needed in the form of test doses to bring the excretion up to normal. In the test dose technique devised by Najjar and Holt [415] 1 mg. of vitamin B₁ is given intravenously and the urinary excretion followed for four hours. Melnick and Field [165], however, advise against this, as the sudden flooding of the organism with vitamin B₁ has a tendency to mask an existing deficiency. Westenbrink and Goudsmit [386] gave test doses of vitamin B₁ parenterally, but found no significant difference in excretion between normal and vitamin B₁ deficient subjects.

Magyar [682, 674], who employs a test dose of 2 to 10 mg. of vitamin B₁ intravenously, states that eighteen to fifty-six per cent. is excreted within twenty-four hours. He considers that if less than eighteen per cent. of the test dose is excreted within twenty-four hours, vitamin B₁ deficiency may be diagnosed. He places no reliance on the estimation of vitamin B₁ in serum as a means of detecting deficiency, since the values range from 1.0 to 15 μ g per cent., with a mean of 7.6 μ g.

The following test has been evolved by Pollack [569] for estimating the level of vitamin B₁ nutrition. A test dose of 1 mg. of vitamin is injected, the urines collected for the four hours following and the vitamin B₁ content determined by the yeast fermentation method (p. 237). The distribution curve of the excretion of 850 persons receiving the test dose showed a definite break at 180 μ g for the four-hour urine. From this and other analyses of the data it was concluded that an excretion of 180 μ g in four hours after the intramuscular injection of 1 mg. vitamin B₁ is the lower limit of normal. Pollack [526] finds that those patients excreting less than 150 μ g of vitamin B₁ after a test dose of 1 gm. usually show some sign of vitamin B₁ deficiency. Using these criteria some twenty-five per cent. of hospital patients were found to be deficient in vitamin B₁.

Mason and Williams [794] state that the test dose of vitamin B₁ should be near the normal physiological requirement, *e.g.*, 1 mg., although they consider that the twenty-four-hour excretion of vitamin B₁ usually gives as much information as the test dose. They consider that the minimal daily requirement of the vitamin is 0.4 mg. per 1,000 calories, and that on this intake the daily excretion (twenty-four hours) averages 119 μ g, with recovery of twenty-one per cent. of a test dose of 1 mg. of vitamin B₁. An excretion of $100 \pm 10\mu$ g or more in twenty-four hours and recovery of at least 20 ± 2 per cent. of a test dose is considered evidence of adequate vitamin B₁ nutrition by Mason and Williams. They observed deficiency symptoms (p. 227) on excretions of 7 μ g to 26 μ g daily and when

one to six per cent. of a test dose was excreted. Similar figures are given by Benson, Witzberger and Slobody [862]. They consider that it is better to estimate the urinary excretion after a diet containing adequate vitamin B₁, *e.g.*, 0.45 mg. per 1,000 calories, than after a test dose of the synthetic vitamin. They recommend the collection of twenty-four-hour specimens of urine on two successive days. The level of vitamin B₁ nutrition is considered adequate if 150 μ g or more of vitamin B₁ is excreted in twenty-four hours or twenty per cent. of the ingested vitamin. This method requires the painstaking collection of urine—only possible in a hospitalized person—and a carefully detailed computation of the vitamin B₁ intake.

THERAPEUTIC USES OF VITAMIN B₁

Attempts have been made to treat many conditions other than those associated with vitamin B₁ deficiency with vitamin B₁. In many cases the treatment has been empirical and not controlled. Much confirmatory work is still needed. In some conditions in which the vitamin has been given there may be a concomitant vitamin B₁ deficiency and administration of the vitamin may have benefited the patient by leading to improvement in appetite, or sense of well-being, and increased strength, without causing any change in the primary condition being treated. It is also possible that vitamin B₁ in large doses stimulates metabolism and has no specific action. In assessing the value of any new remedy the beneficial effects of rest, hospitalization, good nursing and an improved diet are often overlooked. It must also be realized that in many of the conditions in which vitamin B₁ is stated to be beneficial spontaneous remissions often occur and these remissions may coincide with the administration of a new remedy, which gets the credit for any improvement in the patient. Hence the necessity for stringently controlled observations.

Although the daily requirements of vitamin B₁ are put at 1 mg. to 2.5 mg. daily, it is possible by using large doses, *e.g.*, 10 mg. and upwards, to obtain a pharmacological effect. We know that vitamin B₁ is an essential factor in the excitation process of peripheral nerve and that it augments the activity of acetylcholine (p. 170). May not its non-specific effect in the treatment of neurological and "rheumatic" conditions of non-nutritional origin be due to this? Its reputed effects in certain cardiovascular conditions may also be due to its vasodilator action. Vitamin B₁ is absorbed more effectively in small and frequent doses, either orally or parenterally, than in large single doses [414]. It should therefore not be prescribed in large doses for parenteral administration with a long interval of several days between the injections as many drugs are. The bulk of an injection of vitamin B₁ is excreted three hours later [899]. One therefore suspects the value of therapy in which weekly or bi-weekly injections are given. Initially, large doses can be given parenterally, but once the body is saturated small doses at frequent intervals suffice. The oral route is satisfactory if there is no evidence of impaired absorption or utilization (p. 207).

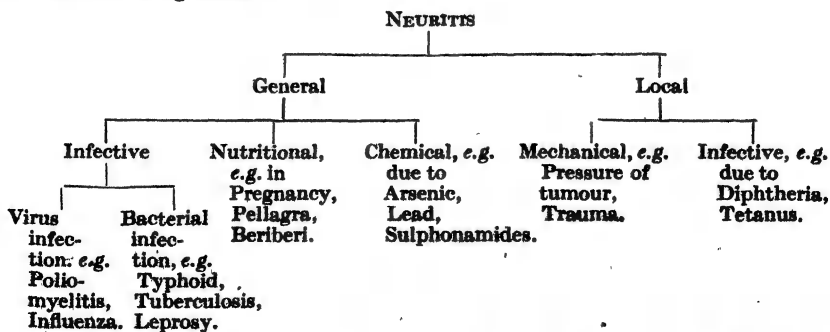
Except in grave cardiac cases of beriberi or vitamin B₁ deficiency there is no evidence that the intravenous administration of vitamin B₁ has any

advantage over intramuscular, subcutaneous or oral routes. Intraspinal administration has been used [191, 396, 444, 697, 888], in doses up to 50 mg. [396, 888]. Such severe reactions have followed this mode of administration that it is not to be recommended [572]. Meningeal irritation has also been observed after the intraspinal injection of doses of 1 mg. to 12 mg. [444, 868].

It has been pointed out that intensive treatment with one member of the vitamin B complex may precipitate symptoms of deficiency in another [767], so that when giving large doses of vitamin B₁, unless there is evidence of an adequate intake of the vitamin B complex, some source of this such as yeast, marmite or wheat germ should be prescribed as well. Aring [868] gives the following scheme of dosage in the treatment of neurological conditions. Vitamin B₁ 25 mg. three times a day, and nicotinic acid 100 mg. six times a day (or 100 mg. nicotinamide) are given parenterally in the early days of therapy. When the desired result is obtained oral therapy is commenced, and if improvement is satisfactory maintenance doses of 2 mg. vitamin B₁ and 20 mg. of nicotinic acid are given. If improvement is going to result it should be observed in a few days, although if degenerative changes have occurred it may be necessary to continue treatment for a long period.

Therapeutic effects resembling those of vitamin B₁ are also obtainable with cocarboxylase [416, 552]. Spies [558] and his collaborators gave intravenous injections of 50 mg. to pellagrins with beriberi, and they observed that the B.B.S. decreased and that the pathological signs in the peripheral and cranial nerves became less conspicuous or disappeared. A beneficial effect was observed within one to four hours and improvement continued for from one to four days afterwards. A psychoneurotic syndrome recognized in the pellagrins responded promptly to the administration of cocarboxylase. Controls showed no decrease in B.B.S. and no improvement in neurological and psychoneurotic symptoms.

Vitamin B₁ Therapy in Neurology. Neuritis. Neuritis may be generalized or localized. The principal causes of neuritis are summarized in the following table :—



Although vitamin B₁ deficiency may result in peripheral or generalized neuritis, no crucial observations have been carried out to determine whether the neuritis secondary to the conditions listed in the table is the result of faulty nutrition. There is no evidence to show that vitamin B₁

plays a direct specific rôle in counteracting virus and bacterial infections, but a number of investigators have reported that the administration of vitamin B₁ relieves the symptoms of the associated peripheral neuritis. The reported relief afforded by vitamin B₁ in some cases of neuritis due to pressure is difficult to explain. Thus Aring and Spies [488] observed that acute pain, due to brachial neuritis following mastectomy, was relieved by daily injections of 50 mg. of vitamin B₁, although it was previously resistant to large doses of codeine and barbiturates (nembutal, phenobarbitone). Since vitamin B₁ prevents and relieves beriberi polyneuritis and since it is essential for the nutrition of the nerve cell, it is possible that its lack or failure to be utilized may play a part in the production of neuritis due to other causes. Many of the deficiency or metabolic diseases that may give rise to neuritis are related to disturbances of nutrition, and it is possible that lack of vitamin B₁ plays a rôle. Such metabolic diseases are often associated with loss of appetite, defective gastro-intestinal absorption, or vomiting. It is possible that the rapid response to vitamin B₁ therapy in some cases of neuritis may be explained on a humoral basis, following the discovery that vitamin B₁ enhances the effect of acetylcholine in the transmission of the nervous impulse (p. 170).

The original work on the treatment of neuritis with vitamin B₁ was carried out in 1935 in America by Vorhaus, Williams and Waterman [271], and by Russell [484] in this country in 1936. The American workers reported on the result of a hundred cases of neuritis of varying ætiology treated with 10 mg. of vitamin B₁ per day orally. There were forty-one cases of "metabolic" neuritis, twenty of infectious neuritis, twenty-two of localized neuritis, eleven associated with pernicious anæmia, and three associated with pregnancy. An analysis of the results showed that forty-four per cent. were permanently relieved of symptoms and forty-eight were improved. Russell treated polyneuritis associated with diarrhœa and vomiting, chronic alcoholism, subacute combined degeneration, and anorexia with parenteral injections of small doses (0.8 to 1.6 mg.). On the Continent some of the earliest work was done in 1936 by Sciclounoff and Broccard [485], who treated fifteen cases of neuritis with 5 to 10 mg. a day for several weeks. Twelve, in whom the neuritis was due to alcoholism, diabetes and diphtheria, showed rapid cure or improvement.

The largest series of cases of polyneuritis treated by vitamin B₁ therapy was studied by Vorhaus [486], who gives the results obtained in 520 cases, over a period of five years. The dosage ranged from 8 to 10 mg. a day for an average time of nine weeks. No improvement occurred in fifteen (three per cent.), partial improvement occurred in 189 (thirty-six per cent.), and freedom from symptoms resulted in 316 (sixty-one per cent.). There was no significant difference in the response to vitamin B₁ therapy whether the neuritis was due to poisoning by heavy metals, or was of toxic, gestational, nutritional, infectious, metabolic, or unknown origin. The 505 cases which were improved after a nine weeks' course of treatment were kept under observation and 315 (62.5 per cent.) had one or more recurrences. Of 170 patients observed up to one year after discontinuing treatment, twenty-one per cent. showed recurrences. 111 patients were followed up for from one to two years and seventy-one per cent. had

recurrences. The recurrence rate reached ninety-six per cent. in a group of 128 followed for over three years. Readministration of vitamin B₁, however, was highly effective in recurrent cases. Improvement usually set in within three weeks and reached a maximum in nine. Vorhaus believes that some individuals may have a greater normal need for vitamin B₁ than can be obtained from normal diets and coins the expression "primary hypothyriamiosis" for this condition. Aring and Spies [488] have treated a number of cases of neuritis associated with pellagra, pernicious anæmia, alcoholism, tuberculosis and pregnancy, and to obtain results they state that large doses, in the neighbourhood of 50 mg. twice a day for at least a week, are necessary. Pain is greatly relieved within a day, and the strength, accuracy, and speed of movement is usually improved within three days, this improvement being possibly due to the relief from pain, which is said to be abrupt and dramatic. The patient, however, quickly reaches a stage beyond which vitamin B₁ will not cause further improvement and residual changes may persist indefinitely.

Kalaja [755] has treated fifty-one cases of neuritis of varying ætiology with 50 mg. vitamin B₁ daily parenterally for seven to fourteen days. Of these thirty-eight suffered from polyneuritis; twenty-seven were relieved in one to ten days, although cases with disturbances of sensation and altered reflexes took longer to return to normal. The cases of neuritis that responded were idiopathic or associated with infections, diabetes, pregnancy and alcoholism; nerve pains associated with herpes zoster, tabes and nervous complications of pernicious anæmia were not relieved. Kalaja concludes that vitamin B₁ is only effective in relieving nervous symptoms due to a deficiency of vitamin B₁, which was present in all the cases that responded to vitamin B₁ therapy. This is also the opinion of Göth [864].

Infective and Bacteriotoxic Neuritis. *Herpes Zoster.* Neuritis following such virus diseases as herpes zoster and poliomyelitis has been treated by means of vitamin B₁ therapy. The prominent feature in herpes is a neuritis with degenerative changes. Goodman [888] treated five cases with daily injections of 10 mg. of vitamin B₁ until the pain disappeared, and he considers that the lesions clear up more promptly than with local treatment. Six to seven injections were given.

On the other hand, Rattner and Roll [489] treated sixteen cases with vitamin B₁ but obtained relief in only two. Roch, Sciclounoff [440] and Barassi [441] have recorded cases of herpes which cleared up after the third injection of vitamin B₁. Gordon [688] found that vitamin B₁ was effective only when given with other standard remedies, although neither were successful alone. Smith [746] reports three cases of herpes zoster in which relief resulted from the injection of vitamin B₁ in doses of 10 mg. directly into the diseased dermatome. The effect might have been non-specific or mediated through changes in the physiology of nervous conduction (p. 170).

Poliomyelitis. It has been claimed that the æstival epidemics of poliomyelitis may be due to some unknown climatic factors and to a lack of vitamin B in the autumn [442, 448]. Helms [744] has put forward the for which she reviewed an extensive literature, that vitamin B₁

deficiency is a predisposing cause of poliomyelitis. She finds indirect evidence in the fact that the disease occurs more frequently when the demands for the vitamin are heavy, as in childhood, pregnancy and after excessive muscular exercise in children. Thirty-five patients were given ten injections of 10 mg., but no conclusion could be drawn whether the treatment was effective or not.

The apparently anomalous observation has been made that mice kept on a diet deficient in vitamin B₁ have an *increased* resistance to the poliomyelitis virus (Lansing strain) [904]. The interpretation of this is difficult, but it is possible that the general metabolism of the animal is upset by lack of vitamin B₁ and that this and possibly other growth factors are not available for the virus which needs them for its reproduction (*cf.* mode of action of sulphonamides, p. 182).

Bauke [444] has treated forty cases of poliomyelitis with intraspinal injections of 10 mg. of vitamin B₁, after removing 5 c.c. of cerebrospinal fluid. Doses of 50 mg. were also tried, but they produced signs of meningeal irritation. Although the results were inconclusive, Bauke records that in three children who had reached the paralytic stage there was an immediate improvement. Gismondi [745] also describes the successful treatment of eleven children with poliomyelitis with daily injections of 2.5 mg. of vitamin B₁.

Stone [865] treated eleven patients suffering from poliomyelitis with vitamins B and E and fever therapy. Five patients received 20 to 50 mg. of vitamin B₁ intraspinally as well as fever therapy. Relief of pain and spasm, improvement in circulation, prevention of contractures, improvement in strength of the affected muscles were reported. Stone admits that the action of the vitamin B₁ is probably non-specific and is due to improvement in cell metabolism and relief of local vasospasm in the extremities. McCormick [866] also describes rapid relief of pain, tenderness and rigidity in four patients treated with 50 mg. of vitamin B₁ daily. Most of these reports are based on very few and uncontrolled cases. The effect, if any, was certainly non-specific as the vitamin B₁ excretion in children with poliomyelitis does not differ significantly from that of normal children [867].

During a poliomyelitis epidemic at Frankfurt-on-Main in 1939 Windorfer [445] treated 150 patients by means of (1) serum, (2) vitamin B₁, (3) complete rest. Thirty-five patients were given ten injections of 10 mg. vitamin B₁. In fifteen a gradual improvement set in, in nine some improvement occurred, and in the remaining eleven no improvement was observed.

Diphtheritic Paralysis. Attempts have been made to treat post-diphtheritic neuritis with vitamin B₁. Feige [447] in 1936 and 1937 observed that paralysis developed in 100 cases. Sixty of these cases were selected, and thirty of them treated with vitamin B₁, the remaining thirty acting as controls. A comparison of the two groups revealed that in those patients receiving vitamin B₁ the paralytic symptoms persisted on the average for thirty days, whereas in the control group they lasted on an average for forty-nine days. Treatment consisted of 1 mg. t.d.s. by mouth and intramuscular injections of 10 mg. on alternate days. Favourable results were also reported by Strnad [448], who treated five cases of toxic

neuritis accompanied by severe myocarditis with injections of vitamin B₁.

Arguing that an acute infectious illness may reveal a latent vitamin B₁ deficiency, Paul v. Kiss [449], of Budapest, used the vitamin in certain nervous conditions in children. He states that the most striking results were obtained in three cases of post-diphtheritic paralysis, which were improved with daily injections of 10 to 20 c.c. of twenty per cent. glucose solution containing from 2 to 20 mg. vitamin B₁. A start was made with 2 mg. and increased until the maximum effect was noticed. Prompt clinical recovery was stated to occur in each case within a few days. Kiss also believes that vitamin B₁ is beneficial in cases of chorea minor, encephalitis, and poliomyelitis. Frey [450] states that vitamin B₁ is of particular value in post-diphtheritic diaphragmatic paralysis. The vitamin was given in doses of 2 mg. intravenously or intramuscularly, once or twice or even more daily, for a total dosage of from 8 to 480 mg. Eighteen cases were studied, and remarkably rapid results were claimed in some. Seven died of heart failure, but even in the fatal cases there was evidence of improved diaphragmatic movement before death.

An account of a personal experience of vitamin B₁ is given by Reich [451], who himself suffered from paralysis following an attack of diphtheria. The arms, legs, diaphragm, and the soft palate were affected. Intravenous injections of 2 mg. had no effect, but increasing the dose to 10 mg. every other day produced subjective and objective improvement. It was found that after an injection of vitamin B₁ there was a definite lowering of the stimulation threshold when the peripheral nerves were stimulated galvanically or faradically. Reich recommends a routine treatment of massage, electricity and vitamin B₁ injections. Tecilazic [452] has treated forty-one cases along the same lines. Five severe cases of diaphragmatic and palatal paralysis were given intraspinal injections of vitamin B₁; two recovered after three or four injections and three died. In the remaining thirty-six cases, which were examples of palatal paralysis, paralysis of accommodation, and loss of reflexes the administration of vitamin B₁ had not the slightest effect. Favourable results, however, were obtained when the vitamin was given prophylactically in doses of 10 mg. a day intravenously in twenty-four cases of malignant diphtheria, and in thirty-three cases of extensive faucial diphtheria. Paralysis either did not occur or its severity and duration were diminished. Only one fatal case was recorded, an incidence of 4.2 per cent., against 14 per cent. in a control series not receiving vitamin B₁.

Donovan and Bannister [707] conclude from controlled experiments that vitamin B₁ has neither a prophylactic nor a therapeutic effect on diphtheritic paralysis or the cardiovascular complications of diphtheria. They state, however, that the treatment gave the patients a sense of well-being and an improvement in appetite, although they doubt the value of the former in the acutely ill case as it tends to encourage restlessness.

Experiments on guinea-pigs were made by Dieckhoff [453], who found that the daily injection of 0.2 mg. vitamin B₁ had no prophylactic or curative effect on animals suffering from post-diphtheritic paralysis. In thirty-seven cases of diphtheria in children who were given 2 mg. vitamin B₁ daily and serum, the incidence of post-diphtheritic paralysis

was higher than in a hundred cases receiving only serum. The blood vitamin B₁ in nine cases of severe toxic diphtheria was 5.7–8 μ g per 100 c.c., which is within normal limits (p. 238). Reinhard and Schwartz [454] found a decreased urinary excretion of vitamin B₁ in diphtheria complicated by paralysis, but they failed to observe any appreciable improvement in paralytic cases receiving the vitamin.

"*Rheumatism.*" Another infection that may be complicated by neuritis is "rheumatism." Borson [414] found that in a group of twenty-eight rheumatoid arthritis patients twenty-five showed signs of vitamin B₁ deficiency as judged by the test dose method (p. 239). Roch and Sciclounoff [440], Keys [455], Muether [456] and others have found that the administration of 1 to 5 mg. vitamin B₁ by mouth or intramuscularly is of help in relieving the neuritic pains of infective arthritis, although they agree that the vitamin has no specific action and lack of it is not aetiological responsible. One of the authors has treated a large number of cases of rheumatism with vitamin B₁ without success.

Acrodynia. Recently attention has been focussed on the therapeutic use of vitamin B₁ in the treatment of acrodynia (pink disease). It is possible that the affected child needs vitamin B₁ in excess of normal requirements, or that difficult feeding may render the reserves of the vitamin low. Forsyth [516] found that four cases of acrodynia improved while receiving supplements of vitamin B₁ (0.5 to 2 mg.) by mouth, and success in the treatment of the disease by administration of the vitamin is also claimed by Durand [517], Williams [518], Gretton-Watson [519], and Aubrey [520]. Durand and his co-workers gave 6 mg. of vitamin B₁ intramuscularly daily for six days, and within three days marked improvement was noted. A course of oral therapy was begun, but relapse occurred, and the child was then placed on 6 mg. parenterally every second day for six more doses. A third series of injections given a month later resulted in practically complete disappearance of all the manifestations of acrodynia. Other workers have given doses of from 2 to 5 mg. a day or every other day. Paterson [521A] gave five patients 3–15 mg. once or twice a week for three weeks, but only obtained improvement in one case. The administration of large doses of vitamin B₁ at intervals of a week or even every other day is unsound, as the vitamin is readily excreted (p. 176). Smaller doses given at least once a day are more likely to give satisfactory results than a big dose occasionally. Vitamin E has also been used in the treatment of pink disease (p. 765).

Leprosy. Leprosy is frequently complicated by neurological lesions such as localized anæsthetic patches, neuritic tenderness and swelling of peripheral nerves and loss of sensation to heat, cold and pain. Attempts have been made to relieve the pain of acute leprous neuritis by means of vitamin B₁ therapy. Badger and Patrick [508] gave daily intramuscular injections of 1 mg. vitamin B₁ to nine severe cases, who also received 2 mg. a day by mouth. In all cases except one pain and tenderness disappeared and the swelling was reduced within three to four days. Treated by other methods the symptoms normally lasted two to three weeks. Villela [509] also noted that relief was obtained in similar cases after forty-five intramuscular injections of 1 mg. vitamin B₁. Larger doses (5 to 20 mg.) were given by

FIG. 50. Acrodynia or pink disease before treatment. A fourteen months old girl, showing wasting, eyes closed to avoid the light, a fretful appearance and skin lesions.

FIG. 51. Acrodynia. The same child as in Figs. 50 and 52 after two weeks' treatment with 6 mg. vitamin B₁ a day parenterally. The general condition has improved, the child no longer avoids the light, the abdomen has filled out and no skin lesions are visible.



FIG. 52. Acrodynia before treatment. A close-up of the hands of the child shown in Fig. 50. They are swollen and cedematous, the skin is desquamating in white flakes, and superficial infection is present around the nail bases.

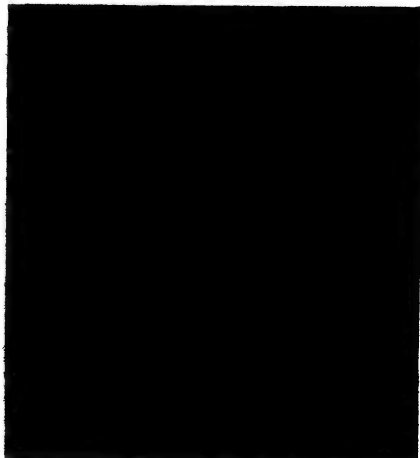


FIG. 53. Acrodynia. The hands of the same child as in Figs. 50 to 52 after two weeks' treatment with vitamin B₁. The swelling has gone and the skin lesions have disappeared, with the exception of a small patch on the right index finger.

Gminder [510] and Keil [511], who record considerable relief from pain in early neuritic cases, as well as a general improvement in the mental and physical condition. According to Hou [512] the vitamin B₁ excretion in lepers is extremely low. As the Eastern leper is under-nourished this is not surprising.

Nutritional or Metabolic Neuritis. There are a number of diseases in which polyneuritis, when it does occur, is directly related to defective nutrition and may be termed nutritional or metabolic neuritis. The better known conditions of this type in which neuritis is encountered are pernicious anæmia, sprue, alcoholism, pregnancy, malignant disease, tuberculosis, diabetes, "chronic progressive" polyneuritis, beriberi and pellagra.

Pernicious Anæmia. About seventy-five per cent. of cases of pernicious anæmia have involvement of the nervous system, and in at least twenty-five per cent. neural or mental symptoms are the first to appear. The neurological lesions associated with pernicious anæmia may be the result of a hypovitaminosis, since they are more common in Western than Eastern Europe, where wholemeal cereals are largely eaten. The achlorhydria and gastritis commonly seen in pernicious anæmia may also lead to defective absorption of the vitamin. Sciclounoff and Naville [457] administered vitamin B₁ in doses of from 4 to 10 mg. for two to three weeks to patients suffering from pernicious anæmia complicated by neurological symptoms. In a series of thirteen, eight showed distinct improvement. Vitamin B₁ has no effect on the blood count. The vitamin has also been used in doses of 5 to 10 mg. by injection with liver in the treatment of the subacute combined degeneration accompanying pernicious anæmia [484, 458-460]. Injections are given daily or every other day for a total of fifteen injections. Aring and Spies [488] state that the neuritis of pernicious anæmia responds to large doses of vitamin B₁ (50 mg. twice daily) given intravenously. Aring [868] has seen no benefit from the use of vitamin B₁ in the treatment of subacute combined degeneration or postero-lateral sclerosis of the spinal cord.

Pellagra, Sprue. The neurological symptoms often associated with pellagra and sprue are generally considered to be due to vitamin B₁ deficiency. Spies and Aring [461] studied the peripheral neuritis of pellagra and demonstrated that it could be relieved with vitamin B₁, while the other symptoms remained stationary or progressed. These observations have been repeated on numerous occasions. The neuritis rapidly disappeared following the injection of 50 mg. vitamin B₁ on two successive days. Campbell and Allison [477] record a case of pellagra with polyneuritis, which cleared up with injections of vitamin B₁.

Alcoholic Neuritis. It was formerly supposed that alcoholic neuritis resulted from the toxic action of alcohol on the peripheral nerves, a view that generally speaking is no longer held since relief is rapidly obtained by the injection of vitamin B₁, even though there is no change in the alcohol consumption [462]. As previously pointed out (p. 216) the chronic alcoholic consumes a diet poor in vitamin B₁; he drinks liquor rather than eats, and the gastritis and anorexia common in the drunkard further restrict the intake of food and hence vitamin B₁, whilst at the same time there is

diminished absorption of what he does eat. Jolliffe, Colbert and Joffe [266, 267] estimated the vitamin B₁ and calorie intake of forty-two chronic alcoholics and reached the conclusions that vitamin B₁ deficiency is the primary cause of the neuritis, and that the improvement varies directly with the vitamin B₁ intake up to a maximum dosage. Further studies by Sciclounoff and Broccard [485], Goodhart and Jolliffe [268], and others [468-470] suggest that vitamin B₁ is of value in the treatment of the condition. Treatment consists of the gradual withdrawal of alcohol if possible, a diet rich in vitamin B₁, and parenteral injections of 10 to 20 mg. or more of the vitamin daily. Aring and Spies [488] believe that to be effective in any neurological condition vitamin B₁ must be given in large doses of at least 50 to 100 mg. a day, which is administered parenterally in several doses of 10 to 20 mg. each.

Only a limited number of patients were treated in each of these series. A large scale clinical trial to determine the value of vitamin B₁ in alcoholic neuritis was made by Brown [562] at the Boston City Hospital, where 286 patients were studied over a period of eighteen years. The average time spent by 118 patients in hospital on orthodox diet and treatment was compared with the same number given special vitamin therapy, which included vitamin B₁, substances rich in the vitamin B complex and liver. The group receiving vitamin therapy were not discharged from hospital as relieved or improved any sooner than those receiving the usual hospital diet and treatment. Ability to walk was the main criterion used as the rest of relief from alcoholic neuritis. Aring [868] also regards the treatment of alcoholic neuritis with vitamin B₁ as disappointing. He has been able to relieve the aching, boring stabbing pain, but has not been able to secure any further relief with vitamin B₁. Aring thinks that in these cases another factor in addition to malnutrition is at work producing the neuritis, although he believes that some common disorder of metabolism underlies all types of polyneuritis, despite the apparent multiplicity of the precipitating agents.

Pregnancy Neuritis. Pregnancy neuritis may be classed as a neuritis due to a nutritional deficiency. An anatomical examination of the affected nerves in pregnancy neuritis shows degenerative changes, most marked in the lumbar region, and consisting of loss of Nissl substance, swelling of cells, eccentricity of nuclei and cell necrosis [869]. During pregnancy the vitamin B₁ requirements are considerably increased (p. 189). Van Coevorden [168] showed that vitamin B₁ deficiency is common in pregnancy, and that in deficient subjects neuritic changes were present in twenty per cent. Schultze [264] observed sixty cases of pregnancy neuritis in 750 pregnant women. Claims have been made that the condition can be relieved by the administration of vitamin B₁ [262-265, 501, 558, 614, 870]. The diet given is rich in vitamin B₁, and in addition parenteral injections of the vitamin in doses of 10 mg. are administered until relief is obtained. McGoogan [870] claims to have obtained good results with injections of 50 mg. to 100 mg. daily. Neuweiler [171] obtained variable results with vitamin B₁. He records success in some cases but others failed to show improvement on doses of 4 to 10 mg. parenterally.

Gastrogenous Neuritis. Gastrogenous neuritis, due to defective absorp-

tion of vitamin B₁ owing to gastro-intestinal disturbances (pp. 208-210), has been treated by means of the vitamin. Cases are recorded by Laurent and Sinclair [259], Ungley [260] and Scott [261], of neuritis following gastric ulcer, pyloric stenosis, cardiospasm, dysphagia, and neoplasms in the gastro-intestinal tract which interfered with the intake or absorption of vitamin B₁. The neuritis responded to parenteral administration of vitamin B₁.

Pressure Neuritis. Neoplastic disease may be associated with neuritis, which may result from defective nutrition or from pressure of a neoplasm on a nerve. Neuritis due to this cause has been relieved by injections of from 10 to 50 mg. of vitamin B₁ [191, 479, 480]. Davis and Bauer [986] have shown that there is evidence of some degree of vitamin B₁ deficiency in patients with neoplastic disease.

Neuritis due to Tuberculosis, Diabetes. It is thought that tuberculosis, senility and diabetes also interfere with the absorption and utilization of vitamin B₁ and may be accompanied by neuritis. Aring and Spies [438] have satisfactorily treated the neuritis in tuberculous patients by dietetic means and by large injections of vitamin B₁ (50 mg.). There are several reports on the treatment of diabetic neuritis with vitamin B₁ [322, 435, 436, 500]. Vitamin B₁ deficiency may occur in diabetics, especially when on restricted diets, but what percentage of the cases of diabetic neuritis have vitamin B₁ deficiency cannot easily be estimated. It is possible that local ischaemia resulting from sclerosis of the arteries may predispose to some of the neuritic manifestations; other possible factors are diabetic polyuria, which may cause an increased urinary excretion of vitamin B₁ (p. 212), and an increased vitamin B₁ requirement owing to increased utilization of carbohydrate following the use of insulin.

Jolliffe [708], who estimates that the incidence of peripheral neuropathy in diabetics is just over two per cent., claims to have relieved the condition in eight diabetics out of nine by the administration of 10 mg. of synthetic vitamin B₁ by mouth, without otherwise changing the diet or therapy.

Needles [871] on the other hand claims that in diabetic neuritis the metabolism of vitamin B₁ does not vary significantly from the normal. He studied seven cases of diabetic neuritis before and after therapy with vitamin B₁, which was given in doses of 10 to 15 mg. daily for five and a half months. In contrast to Jolliffe and others, Needles observed no improvement in any of the cases; in fact two became worse under treatment. A possible explanation is that in Needles' cases there was severe neurological involvement and that possibly the damage was irreversible and irreparable as it may be in severe subacute combined degeneration of the spinal cord in pernicious anaemia, in which liver therapy is not always successful.

Toxic Neuritis. Neuritis may be due to exogenous chemical toxins, but as yet there is no conclusive evidence that a lack of vitamin B₁ plays a major rôle in the development of the "chemical" neuritides. It has been reported that the administration of vitamin B₁ brings relief of neuritic symptoms in poisoning by such toxic agents as tobacco [492], arsenic [481, 482], lead [483], mercury [498], thallium [485, 486], carbon disulphide [487],

and sulphonamide derivatives such as uleron [488, 489] and sulphacetamide (albugid) [490].

The effect of large doses of vitamin B₁ on the toxicity of sulphapyridine, sulphathiazole and Irgamid (N-dimethylacroylsulphanilamide) has been investigated by Fleisch and De Preux [894], who state that it diminishes the degree of albuminuria and hæmaturia, two well-known toxic symptoms of these sulphonamides. Vitamin B₁, however, had no effect on the mortality rate in experimental animals and on the bradycardia produced by these drugs. It is possible that the effect on the albuminuria and the hæmaturia was due to the diuretic action of vitamin B₁ (p. 265). Fleisch and De Preux conclude that the toxicity of the sulphonamides cannot be attributed to a state of vitamin B₁ deficiency induced by the drugs. Higgins [900] has noted that the toxic symptoms normally noted with the sulphone promin (promanide), at present under clinical trial for the treatment of tuberculosis, are prevented in rats if they are given six times the normal allowance of vitamin B₁, riboflavin and vitamin B₆. The toxic effects include hyperæsthesia, loss of weight, anorexia, lassitude, irritability and an anæmia said to be hæmolytic, but probably due to iron deficiency. Yeast, a rich source of the vitamin B complex, failed to diminish the toxicity of promin in guinea-pigs [901].

As there is evidence that vitamin B₁ may be synthesized in the human intestine, and that this synthesis is inhibited by the bacteriostatic activity of the sulphonamides [780], it would seem advisable when giving these drugs to administer supplements of vitamin B₁ and the B vitamins.

Porter [481] observed improvement in a few days in a case of arsenical neuritis, in which a dose of 2.4 mg. was injected daily. Aring and Spies [433] record a case of neuritis due to emetine, which responded within twelve hours to intravenous injections of 100 mg. vitamin B₁ twice daily. Large doses of 100 to 500 mg. a day were also given by mouth. They found that vitamin B₁ was ineffective in two cases of neuritis due to triorthocresyl phosphate poisoning (Jamaica ginger paralysis), in which doses of 20 mg. and 500 mg. respectively were given by mouth for a month. Negative results were also obtained in a case of neuritis attributed to methyl chloride poisoning.

Localized Neuritis. Relief of localized neuritis, which is usually of infectious or mechanical origin, has been reported by several investigators. Among the commoner neuritides that have been treated are sciatica [440, 494-499], sacro-iliac, intercostal and shoulder girdle neuritis, ulnar and crural neuritis [502-507]. The number of patients in the reported series is often small, and in general the studies have not been controlled. The evaluation of such reports is therefore a difficult matter. Many of these conditions also clear up spontaneously with rest and general treatment.

Coste and Metzger [502] have used repeated injections of 4-20 mg. of the vitamin to alleviate pain in a number of cases of neuralgia and arthralgia of various origin. Among the conditions relieved were cervico-brachial neuralgia, peri-arthritis, and chronic rheumatic coxitis.

Peripheral Nerve Trauma. Observations on the effect of vitamin B₁ on the post-traumatic regeneration of peripheral nerves are scanty and inconclusive. Gastaldi [876] concluded that vitamin B₁ deficiency had

little effect in influencing the post-traumatic regeneration of peripheral nerve in pigeons. On the other hand, Tangari [877] following the course of regeneration of the sciatic nerve of rats after section, records complete and permanent paralysis in the rats with vitamin B₁ deficiency, two-thirds of them showing trophic sores. Animals receiving injections of vitamin B₁ were stated to show acceleration of motor and sensory recovery; histologically the results were not convincing. Similar observations were made by Müsch and Rössel [878], who crushed or severely injured the sciatic nerve of guinea-pigs; paralysis of the extremity and trophic lesions occurred. Motor function recovered in eighteen to twenty-eight days in animals treated with vitamin B₁ (12.5 mg. daily) whereas in the untreated cases trophic sores developed in four to eight days. There was no evidence that the administration of vitamin B₁ had any significant action after the division of the nerve. Clinical observations were made by Demel [879], who found that 8 mg. of vitamin B₁ daily by injection had a beneficial effect on motor disturbances in four cases of section of the median and ulnar nerves showing incomplete recovery after nerve suture. Sliosberg [515] reported good results with injections of 10 mg. of vitamin B₁ up to a total of fifty injections in the relief of pain in eighty-three painful amputation stumps. Complete relief occurred in eleven cases; considerable improvement in fifty cases; medium improvement in twelve cases; little improvement in seven cases; and no relief at all in three cases. Apart from this very few details are given so that it is difficult to draw any definite conclusions. Summarizing these observations it may be said that a case has possibly been made for the beneficial effect of vitamin B₁ in traumatic lesions of peripheral nerves, provided the nerve has not been severed.

Affections of the Cranial Nerves. Attempts have been made to relieve the severe pain of trigeminal neuralgia (tic douloureux) by means of vitamin B₁. Vorhaus [523] studied five cases which had been operated on and showed post-operative recurrences. Three showed complete relief of symptoms, and two showed some improvement. Seven cases were treated by Bakhsh [524] with daily intramuscular injections of from 6 to 80 mg., the total quantity given to each patient being from 84 to 210 mg. Dramatic relief was stated to have occurred in four cases, two experienced partial relief, and one was unaffected. The satisfactory treatment of isolated cases is described by other authors [527]. The experience of Borsook, Kremers and Wiggins [528], who reported on the treatment of fifty-eight patients suffering from trigeminal neuralgia, suggests that the results are best if 10 mg. of vitamin B₁ is injected daily for a long time, with liver extract three times a week. They also prescribed a high vitamin and low carbohydrate diet with a rice polishings concentrate providing 5 mg. of vitamin B₁ daily. The fifty-eight cases were under observation for from six to fourteen months, and thirty-seven were "markedly improved," fifteen improved, three slightly improved, and three unchanged. But Borsook and his fellow-workers point out that the treatment must be continued for as long as six months before the maximum benefit is obtained. This is also the opinion of Borson [414], who found that the vitamin B₁ excretion in a group of patients with trigeminal neuralgia indicated

moderate to mild degrees of vitamin B₁ deficiency. He considers that there is a definite indication for the use of vitamin B₁ as a therapeutic measure. Rose and Jacobson [706] conclude that vitamin B₁ has no specific beneficial effect on the condition. As in many nervous conditions the pain of trigeminal neuralgia shows so many spontaneous remissions that the assessment of any new form of therapy is difficult.

Affections of other cranial nerves that have been treated by vitamin B₁ therapy include facial paralysis, nerve deafness and optic neuritis [582, 588]. Lerner [529] describes seven cases of facial paralysis, all of which were cured by intramuscular injections of vitamin B₁ in doses of from 2 to 10 mg. daily, over a period of six to eighteen days. Cases of nine and fourteen years' standing were stated to have shown some improvement.

According to the observations of Selfridge [530] six patients with deafness associated with lesions of the eighth nerve improved after the injection of vitamin B₁ and nicotinic acid. Although the improvement was greater after nicotinic acid, it was thought that the effects of the two vitamins were sometimes additive. Seven cases of deafness were treated with vitamin B₁ by Veasey [531], who found that the results varied, but suggested that vitamin B₁ might be of some value in nerve deafness. Vitamin B₁ is also stated to give relief in some cases of tinnitus [658, 659]. The successful employment of vitamin B₁ therapy in neuritis of the eighth cranial nerve is described by Brandenburg [578]. The patient, who suffered from severe bilateral tinnitus and impairment of hearing following irradiation therapy, experienced relief after the daily injection of 10 to 15 mg. of vitamin B₁ for nine days. Childrey [681] used vitamin B₁ in doses of 10 to 15 mg. daily, and also the vitamin B complex, in the treatment of non-specific deafness, but he states that very few patients felt that they were benefited, and that audiometer tests confirmed that there was no improvement. He also used it in several cases of tinnitus, with relief in only one case. Using audiometer tests, Shambaugh and Jennes [875] were also unable to confirm the value of vitamin B₁ in large doses (50 mg.) in the treatment of nerve deafness and tinnitus aurium. They state that many of the claims are based on the subjective evidence of patients and are insufficiently controlled. The evaluation of therapy for the improvement of hearing must be based on repeated audiometric tests, and to be significant there must be a sustained improvement of at least 10 decibels above the pre-treatment level. A review of the literature suggests that there is very little critically controlled evidence in favour of the use of vitamin B₁ for the alleviation of deafness and tinnitus.

Harris and Moore [544] reported on twenty cases of Menière's syndrome treated orally with 250 mg. nicotinic acid and 20 mg. vitamin B₁ daily. Seventeen became entirely free from vertigo, and the remaining three showed marked improvement in the severity and number of attacks. It is pointed out, however, that treatment must be carried out for two to three months to obtain the full effect. Atkinson [829] also describes the treatment of nineteen cases with vitamin B₁ and nicotinic acid. Ecker and Woltman [545] state that large doses of vitamin B₁ and C have effected a cure in some cases of Wernicke's encephalopathy. Jolliffe and his

colleagues [188, 742, 743] have demonstrated that the oculomotor palsies of this syndrome are a manifestation of vitamin B₁ deficiency and respond to treatment with the vitamin; they also state that the stupor may respond, but not the ataxia. Large doses, e.g., 50 to 100 mg. t.d.s. for a week are recommended by Jolliffe [933]. Aring [868] states that too much must not be expected of vitamin therapy in the treatment of Wernicke's encephalopathy and that it is unusual for the associated mental deterioration to improve after administering vitamin B₁. In his opinion many cases may recover apparently spontaneously.

Diseases of the Spinal Cord. Disseminated Sclerosis. Reports on the value of vitamin B₁ in disseminated sclerosis are inconclusive. Thus Molnar [508], Böhm [534] and Bakhsh [524] obtained no improvement; Grewin [683] observed "slight or no improvement in chronic cases"; whereas Stern [518] recorded considerable improvement in cases receiving 10 to 100 mg. intrathecally. Of the seven cases described by Roch and Sciclounoff [440] and treated with vitamin B₁ in doses of 5 to 10 mg. daily, four were stated to show definite improvement. These investigators, however, are careful to state that "this may perhaps have been due to a natural remission in the disease and to hospitalization rather than to our interventions." Five cases of advanced disseminated sclerosis were treated by Moore [535], who gave intravenous or intramuscular injections of 60 to 160 mg. of nicotinic acid and 33.2 mg. vitamin B₁ two to three times weekly. Considerable subjective and objective improvement but not complete remission was observed. When treatment was discontinued there was a return of spasticity and lack of co-ordination. The vitamin B₁ excretion in a group of fourteen patients suffering from disseminated sclerosis was examined by Borson [414] and found to be very low. Four had some symptomatic improvement from the use of vitamin B₁. The claim has also been made that vitamin B₁ and adenosine triphosphate (p. 109) improved cases of disseminated sclerosis [880]. Masek [536] treated fifty-eight cases of diseases of the peripheral and central nervous systems with daily injections of 10 mg. of vitamin B₁. All the peripheral cases responded to treatment, but the central cases, including subacute combined degeneration, disseminated sclerosis, and meningo-radicularitis were resistant.

Tabes. Wintrobe, Miller and Lisco [881] have been able to produce spinal cord lesions in pigs similar to the lesions of tabes dorsalis in man by feeding diets deficient in the vitamin B complex. Clinically it has been recorded that vitamin B₁ relieves the lightning pains of tabes. Its use is based on the supposition that two factors are at work in the production of the syndrome: one the *Treponema pallidum*, the other a nutritional deficiency.

Metildi [537] treated six tabetic patients, in whom antisyphilitic treatment had been ineffective, with intramuscular or intravenous injections of 10 mg. vitamin B₁. Relief of pain, complete or in part, was reported in a few days in four patients. Reese and Hodgson [538] state that when patients with tabes were given large doses of liver extract, vitamin B₁ and yeast for two weeks without antisyphilitic treatment, remarkable improvement occurred, especially in the ataxia, sensory power and visual fields.

They consider that the focal symptomatology of neurosyphilis may be due to regional vitamin depletion. Aring and Spies [488] administered 100 mg. vitamin B₁ intravenously during tabetic crises to six patients, with the result that relief was obtained. In all cases in which pain returned physiological saline alone was injected, and the results were just as good as with vitamin B₁. Twelve out of a group of fifteen patients suffering from syphilis of the central nervous system were shown by Borson [414] to be remarkably deficient in vitamin B₁. Stone [689], who has worked on the subject since 1939, has treated sixty-three tabetic patients with weekly intraspinal injections of vitamin B₁ in doses of 20 mg. for children, and 50 mg. for adults up to a total of 165 injections. At the same time a vitamin B complex preparation, wheat germ oil and arsenic and bismuth were administered. Some patients also received artificial fever therapy. In seventeen out of twenty-three patients with advanced tabes improvement occurred in gait, bladder symptoms, visual disturbances and lightning pains. The greatest improvement was shown in those receiving fever therapy. Lack of controls and multiplicity of treatments make this work difficult to evaluate. The fever therapy combined with arsenic and bismuth alone may have produced the improvement.

Cochems and Kemp [882] have treated the largest group of tabetics so far with vitamin B₁. They treated twenty-six patients with indisputable evidence of tabes with lightning pains with vitamin B₁ over a period of eight months, giving an average of eighteen injections intravenously and an average dose of 61 mg. Sixty-five per cent. experienced no relief; sixteen per cent. got partial relief; and nineteen per cent. obtained complete relief. Definite proof of the value of vitamin B₁ was lacking. Moore and Woods [595] conclude that the suggested interrelationship between nutritional deficiency and neurosyphilis in the pathogenesis of tabes has some clinical and experimental backing, but that it requires further study before it can be accepted. Little is known of the effects of syphilis on vitamin B₁ requirements, or of the effects of the heavy metals used in anti-syphilitic treatment.

Pernicious Anæmia (Cord Symptoms). Certain residual neural manifestations are apt to remain after the treatment of pernicious anæmia by liver therapy. From a study of nineteen patients Zillhardt [716] and his co-workers concluded that intramuscular injections of 10 mg. of vitamin B₁ three times a week for two months had a beneficial effect on the residual neurological signs and symptoms of pernicious anæmia that seemed stationary in spite of persistent intensive anti-pernicious anæmia therapy. Continued use of vitamin B₁ after two months produced no further change. It was thought that vitamin B₁ intramuscularly in the dosage stated was more effective than 3 mg. by mouth twice a day. Controlled studies with a vitamin B complex preparation containing vitamin B₁, riboflavin, nicotinic acid, vitamin B₆ and the filtrate factor, showed that this had very little effect when given intramuscularly three times a week [717]. The cord symptoms in pernicious anæmia are stated to be relieved by large doses of vitamin B₁ [577, 628], although Aring [868] has been unable to verify this.

Landry's Paralysis. Windorfer [445] treated fifteen cases of Landry's paralysis with intrathecal injections of vitamin B₁ and the impression was

obtained that this form of therapy was of some value. The final results—three deaths, ten serious and permanent paralyses and two recoveries—are inconclusive. Roberts [446] also treated a case of Landry's paralysis with 4 mg. of vitamin B₁ by injection for ten weeks in addition to the usual measures, but failed to obtain any noticeable improvement. Villarets [676] and his co-workers claim to have treated a case of Landry's paralysis of alcoholic origin with intraspinal injections of 30 mg. and 20 mg. intravenously. In three weeks a total of 150 mg. and 980 mg. were given by these routes respectively as well as 980 mg. subcutaneously—a total of 2·01 grams—and the patient is stated to have rapidly improved.

Other Diseases of the Nervous System. McCormick [540] states that he has given large doses of vitamin B₁ with good results in a variety of diseases of the central nervous system, including encephalitis, post-encephalitic parkinsonism, poliomyelitis, progressive muscular atrophy, and pseudohypertrophic muscular dystrophy. Hawke [607] failed to obtain demonstrable improvement in seven cases of muscular dystrophy treated with vitamin B₁ and the vitamin B complex. Some of the patients reported improvement, but they also did so with a placebo. Borson [414] claims to have obtained a remarkable improvement in co-ordination in a case of Friedrich's ataxia receiving 2 mg. vitamin B₁ a day for six months. But he failed to observe any change in a group of patients with miscellaneous diseases of the central nervous system; some in fact got progressively worse. Robie [572] gave intraspinal injections of vitamin B₁ to nineteen patients suffering from chronic degenerative disorders of the central nervous system, but failed to observe improvement in any of them.

Other diseases of the central nervous system that have been treated with vitamin B₁ include amyotrophic lateral sclerosis [541], spastic paraplegia [542] and Adie's syndrome [543]. According to Stone [685] vitamin B₁ and artificial pyrexia is of value in the treatment of Sydenham's chorea or chorea minor, which is an encephalopathy of indefinite origin. Doses of 10 mg. of vitamin B₁ were given intravenously as well as a preparation containing the vitamin B complex.

Relief of Pain. Stern [518] has given intraspinal injections of 10 to 100 mg. of vitamin B₁ for the relief of intractable pain. A hundred and twenty injections were given to twenty-eight patients with inoperable cancer, von Recklinghausen's disease, disseminated sclerosis, thromboangiitis obliterans, pregangrenous conditions, tabes, hypertrophic spondylitis, Paget's disease, osteoporosis of the spine, and other painful conditions. Most of the patients experienced considerable relief from pain, and no harmful results were noted as a result of the injections. There appears to be some danger, however, from the uncontrolled administration of very large doses of vitamin B₁ intraspinally. Aring and Spies [488] were unable to confirm the analgesic action of the vitamin in patients suffering from severe pain (carcinoma, cord tumour, trigeminal neuralgia), in spite of doses of 50–100 mg. given intravenously for ten days.

Baer [514] states that the injection of 10 to 50 mg. of the vitamin produces considerable relief in the local pain after tonsillectomy, and he

has also given injections to relieve acute and radiating pain in otitis media [658]. Krieg [521], Ochsner and Smith [522] have used vitamin B₁ for the relief of pain in varicose ulcers. Krieg gave daily intramuscular injections of from 5 to 10 mg. until improvement was noted, then 1 mg. t.d.s. by mouth for a week, followed by a week's administration of 1 mg. twice daily, and then a week's administration of 1 mg. once daily. He states that not only were pain, stiffness, heaviness and pressure relieved, in from four to eight days' treatment, but in twenty-one out of thirty-five cases a definite decrease in the size of the varicosities was recorded. Eight cases out of ten treated by Ochsner and Smith were relieved of symptoms following the oral administration of 5 mg. of vitamin B₁ t.d.s. They suggest that if the pain does not subside in three to four days the dose should be doubled. The explanation for the relief of pain in these cases is not clear. It is possible that the patients were suffering from a vitamin B₁ deficiency and that the relief was occasioned by correction of the deficiency. There is also some evidence that vitamin B₁ produces peripheral vascular dilatation. This may explain the action of the vitamin in relieving the pain of migraine, as stated by Bändler [627], and Palmer [872], who injects 60 mg. to 120 mg. of vitamin B₁ intramuscularly or intravenously to terminate an attack, and 80 to 90 mg. daily. Palmer's cases were of extreme severity as far as pain and frequency were concerned, and many of them did not respond to treatment with ergotamine tartrate.

Vitamin B₁ has been used in dentistry for the relief of pain of "dry socket" after extraction. Injections up to 100 mg. and 5 mg. a day orally have been given [873, 874].

Psychiatry. Vitamin B₁ has been used in certain mental states, particularly in the psychoses associated with pellagra and of gastric origin. Mindus [546], who stresses the frequency of digestive disturbances in mental patients, selected two groups of thirty cases suffering from "gastrogenic neurasthenia." One group was treated with vitamin B₁, riboflavin and a special diet; the other received only routine psychiatric treatment. The former showed a higher percentage of recoveries, the result being statistically significant. A number of cases of depression were treated by Sopp [547] with injections of 10 c.c. vitamin B₁ and a cholagogue. In eighteen cases out of thirty-two recovery was more rapid than would have been expected under routine treatment. Vitamin B₁ has been used in conjunction with insulin in the shock treatment of schizophrenia. It is claimed that the simultaneous injection of 2 to 10 mg. of vitamin B₁ minimizes the occurrence of convulsions and prolonged coma [548, 550, 556]. Ziskind and her co-workers [740] were unable to confirm this. Their observations are probably correct, as the bulk of the evidence goes to show that vitamin B₁ has little effect in the human on the blood sugar level (p. 167).

Lewald and Alexander [578] considered that lack of vitamin B₁ in the foetus or in early infancy might result in degeneration of the cortical cells associated with the function of intellect. They therefore gave injections of 6.7 mg. at three or four day intervals to eight mental defectives for three months. No significant mental improvement occurred,

although in the case of patients presenting feeding problems there was an improvement in appetite and physical condition.

Alcoholic Psychoses. Vitamin B₁ has been tried in the treatment of alcoholic psychoses. Some alcoholics develop hæmatological or neurological changes, others suffer from psychoses such as Korsakoff's psychosis or delirium tremens. Brodsky [471] treated fifty-three patients suffering from alcoholic psychoses with daily intramuscular injections of 1 to 5 mg. vitamin B₁ and sedatives such as bromide, chloral, or phenobarbitone, the alcohol being withdrawn where possible. Improvement occurred in all cases after periods varying from weeks to months. Cases of acute alcoholic psychoses were treated by spinal drainage and daily injections of vitamin B₁.

Korsakoff's Psychosis. Bowman [472], Champeix [473], Roch and Sciclounoff [440], record the satisfactory treatment of Korsakoff's psychosis with injections of vitamin B₁ (5 to 10 mg. daily). Bowman, Goodhart and Jolliffe [579] studied fifty-one subjects with Korsakoff's psychosis who were maintained on a diet of borderline adequacy in vitamin B₁ for an average of eleven days, during which time 11·7 per cent. recovered. After this control period thirty-six patients were given a diet rich in vitamins and a concentrate estimated to provide 3·8 mg. of vitamin B₁ daily. Twenty patients received in addition 10 to 50 mg. of the vitamin daily by parenteral administration, and these showed an incidence of recovery approximately seven times as great as those treated by diet alone. They failed, however, to confirm this conclusion by statistical analysis and were unable to state whether the increased incidence of recovery in the group receiving vitamin B₁ was due to the form of therapy employed or to spontaneous recoveries.

There is still some considerable doubt expressed by some authorities on the part played by vitamin B₁ deficiency in the causation of Korsakoff's psychosis, which may be encountered after head injury, subarachnoid hæmorrhage, and other conditions unrelated to dietary deficiency. Even when associated with alcoholism recovery may occur without special therapy. It is said that some patients with Wernicke's encephalopathy are left with a residual Korsakoff's psychosis after treatment with vitamin B₁ [938]. In a recent review on the subject Caldwell and Hardwick [934] state that they have obtained beneficial results in Korsakoff's syndrome with prolonged vitamin B₁ therapy.

Delirium Tremens. Kiene [476] and his colleagues advance the view that delirium tremens is not precipitated by the withdrawal of alcohol but to a deficit of vitamin B₁ in the brain parenchyma. Their patients are treated with a high calorie diet containing 1·5 to 2 mg. of vitamin B₁, four ounces of whisky every three hours, and intravenous injections of 25 to 50 mg. of vitamin B₁ twice daily for several days. Recovery from acute symptoms occurred twice as fast as when intravenous vitamin B₁ was given alone. In the treatment of a threatened attack of delirium tremens Kloster [474] gives one to three intravenous injections of 20–80 mg. vitamin B₁ every hour or two. Under this treatment eight out of ten patients slept quietly the first night. In frank cases 30 to 50 mg. were injected intravenously on three occasions, and marked improvement was noted two hours after. Bowman, Wortis and Keiser [475] recommend a

high calorie diet (3,000–4,000 cal.) and vitamins B₁ and C. Caldwell and Hardwick [934] state that large doses of vitamin B₁ have lowered the mortality rate in delirium tremens. Seiclounoff and Flagg [491] found that vitamin B₁ in doses of 10 to 100 mg. intramuscularly was effective in cases of threatened delirium, but was valueless in an actual attack. This was confirmed by Mainzer and Krause [598], who observed that a dose of 550 mg. spread over two days was quite ineffective in one case, although surprisingly enough the patient responded to 0.5 gram nicotinic acid. Rosenbaum [631] states that the course of delirium tremens is not altered by vitamin therapy and Romano [582], reviewing the literature since the nineteenth century, states that the prognosis then was as good as it is now if alcohol is withdrawn and a sedative given.

Vitamin B₁ in Ophthalmology. Several cases of toxic amblyopia (retrobulbar neuritis) have been treated by means of vitamin B₁ or yeast therapy [835, 634–637, 753]. Johnson [635] describes the treatment of five cases of alcohol-tobacco amblyopia with pure vitamin B₁, which was given in doses of 12 mg. a day orally. The patients were all inveterate alcoholics and were no doubt suffering from vitamin B₁ deficiency. Improvement occurred in four of the cases after several weeks' treatment with vitamin B₁ and the withdrawal of alcohol and tobacco. No controlled observations were made, and it may therefore be argued that the withdrawal of the toxic agent contributed towards the success of the treatment. Carroll [634, 938], however, allowed his patients to continue with their accustomed alcohol and tobacco and gave them yeast, yeast and liver extract, cod-liver oil and vitamin B₁. He gives complete case histories of twenty-five patients treated with vitamin B₁ and vitamin B complex products. Vision improved in twenty-one of the patients. The results were claimed to be at least as good as those of twenty-five controls, from whom alcohol and tobacco were withdrawn during treatment. Some of the patients consumed a litre of gin or spirits and ten to twenty cigars a day. Lcinfelder and Stump [752] failed to observe any beneficial effects from giving vitamin B₁ to patients with amblyopia resulting from tryparsamide therapy. Grosz [637] treated fifteen patients with nyctalopia, misty vision, and weakness of the legs with preparations rich in vitamin B₁, yeast, and injections of 10 mg. of vitamin B₁. Only three remained unchanged, the remainder either recovering or showing considerable improvement. Claims have been made that vitamin B₁ is effective in the treatment of optic neuritis [582], non-toxic retrobulbar neuritis [583, 637], keratitis dendritica and corneal herpes [637, 638, 640]. Czukrasz [640] treated corneal herpes with injections of 8 to 10 mg. of vitamin B₁ and an ointment containing 0.03 to 0.1 per cent. vitamin B₁. In nine cases one failure was recorded. It was stated that epithelization began after four to eight days and healing was complete in a fortnight.

Retrobulbar neuritis has certainly been described in association with other lesions in vitamin B complex deficiencies, either actual as in pellagra and beriberi, or conditioned, as in alcoholism, pregnancy, etc. Such cases respond to vitamin therapy. There is no evidence, however, that vitamin therapy has any effect in ophthalmic conditions not resulting from nutritional deficiency.

Vitamin B₁ in Cardiovascular Disease. Generally speaking vitamin B₁ therapy is of no value in cases of cardiovascular disease that are not associated with vitamin B₁ deficiency. Mild forms of deficiency, however, are probably far more common than generally suspected. Studies in human experimental hypovitaminosis B₁ (p. 227) have shown that the cardiovascular signs and symptoms of vitamin B₁ deficiency may appear on diets containing 0.86 mg. of the vitamin per day. Wood [288] points out that hypovitaminosis B₁ should be suspected in patients on restricted or poor diets, and in cases of right ventricular failure of unknown ætiology; and it should be borne in mind when dealing with pregnant women. The therapeutic test may be applied, *i.e.*, improvement sets in after the injection of large doses of vitamin B₁. Wood [288] recommends doses of from 10 to 50 mg. intravenously in severe cases, and 5–10 mg. intramuscularly or orally in milder cases. Bickel [294] gives 50 mg. vitamin B₁ intravenously four times a week or even daily. The dosage is reduced to 10–20 mg. as improvement sets in, and then to two injections of 10 mg. a week. As pointed out before, widely spaced injections are less effective than frequent oral therapy.

Although the cardiopathies due to deficiency disease do not respond to cardiotonics alone, it is often found that they are useful adjuvants to vitamin B₁ therapy. In cases with œdema the treatment should consist of rest in bed, limitation of fluids and salt and the administration of digitalis or diuretics such as mersalyl or neptal. Marvin [588] gives intravenous or intramuscular injections of 50 mg. of vitamin B₁ daily as a routine, and after two or three weeks, when improvement has occurred, he reduces the dosage to 10 to 20 mg. daily. If the symptoms are not particularly severe 80 grams of brewer's yeast daily may be given instead.

Moia and Batlle [585] have used vitamin B₁ in certain cardiac cases without an underlying hypovitaminosis. They state that there is a type of præcordial pain which has a different clinical picture and pathogenesis from those of angina pectoris and coronary occlusion, and which may appear in persons with a normal cardiovascular system or in cardiovascular diseases. It develops predominantly in persons with nervous hyperexcitability. Moia and Batlle treated a group of seventy-eight cases with 10 mg. of vitamin B₁ parenterally every other day for a total of nine to thirteen injections. Most of the patients had no clinical cardiac signs or suffered from hypertension or vascular sclerosis. Pain disappeared completely in forty cases and temporarily in thirty; eight failed to respond. In a group of sixteen cases of mitral stenosis and neuro-circulatory asthenia, pain disappeared completely in five, temporarily in five, and not at all in six. It is possible that any improvement was due to the vasodilator action of vitamin B₁.

Naide [587] states that the administration of 100 mg. of vitamin B₁ intravenously every day for a week or two brought about complete relief of pain in seven patients out of ten suffering from peripheral vascular disease. Partial relief was also experienced by two of the patients, but this form of therapy had no effect on gangrene, ulcers or objective neurological changes in these cases. Maintenance doses of 20 to 100 mg. once or twice weekly were necessary to keep the patients free from pain. Among

the conditions treated were thromboangiitis obliterans, intermittent claudication and ischæmic neuritis. These results may be explained on the ground that defective circulation hinders a normal supply of vitamin B₁ to the tissues, and that in this way their metabolism, already impaired by partial anoxia, becomes sufficiently disturbed to evoke pain. Piotrowski [127] found that vitamin B₁ alone had little effect in cases of intermittent claudication, but caused marked subjective improvement when given simultaneously with acetylcholine. The combined effect of oestrogens and vitamin B₁ on vascular spasm was studied by Herrmann and McGrath [588]. They gave twelve patients with arterial insufficiency, due to secondary vasomotor instability associated with active arteritis, moderate quantities of oestrone and 50 mg. of vitamin B₁ intravenously for five days. Eleven were so improved that they returned to work, and at the end of two years only three patients had had a relapse. Either the vasomotor instability completely disappeared or the disease remained so quiescent that it caused no disability. In the treatment of Raynaud's disease, it is said that recurrence can be prevented after sympathectomy by the administration of vitamin B₁ [589]. Magyar [887] states that large doses of vitamin B₁, *e.g.*, 100 mg. intravenously, cause relief of pain in arteriosclerosis.

Claims have been made that ionization with an ointment containing acetylcholine and vitamin B₁ is effective in the treatment of frostbite, erythrocyanosis, varicose ulcers, eczema, pruritis and neurodermatitis [908]. The observations were not controlled and improvement may well have been due to ionization with acetylcholine alone. There was no evidence that the vitamin B₁ contributed to the reported improvement of the patients.

Vitamin B₁ has been used in conditions affecting the blood forming organs. Thus Massias [590] has used it with pentnucleotide to induce leucocytosis in cases of leucopenia, but neither the reasons for using it nor the case histories given are very cogent. Secher [591] on very slender evidence treats the thrombocytopenia that may follow the administration of gold compounds with injections of vitamins B₁ (2-5 mg.), C (150 mg.) and A (1,500 I.U.). In the absence of disease, injections of 15 to 100 mg. of vitamin B₁ are said to increase the normal thrombocyte count by twenty per cent. [592].

Vitamin B₁ in Gastro-intestinal Conditions. Many gastro-intestinal disturbances have been ascribed on clinical and experimental grounds to vitamin B₁ deficiency. On the other hand, it is more likely that these disturbances prevent effective absorption and so condition vitamin B₁ deficiency. In most cases the deficiency states have been multiple and not pure B₁ hypovitaminoses, and "vitamin B" therapy has often meant the administration of concentrates containing not only vitamin B₁ but also other vitamins of the B complex. It is therefore difficult to assign an exact rôle to the effects of vitamin B₁ in these studies. Where vitamin B₁ deficiency can be incriminated as the cause of anorexia, or is associated with ulcerative colitis, vomiting, diarrhoea, gastrointestinal diets, or impaired absorption then the vitamin should be given in adequate amounts, preferably by parenteral injection if absorption by mouth is debatable.

Löwenstein, Neumann [597] and Borson [414] have shown that many cases of ulcerative colitis and cirrhosis of the liver have a very low vitamin B₁ excretion. The latter is possibly due to decreased ability of the liver to phosphorylate the vitamin for utilization and storage. Shiffer and Ferguson [789] have found vitamin B₁ ineffective in the treatment of idiopathic ulcerative colitis. Cheney [598] has described the dramatic response of thirty-two cases of chronic diarrhoeas of unknown ætiology and cases of mucous colitis to treatment with vitamin B₁. The daily oral dose was from 1.5 to 8 mg.; in some cases it was given parenterally in doses from 6.6 to 10 mg. One case, a nervous woman, had had distressing diarrhoea for two years, and for a year had been treated with the usual bland diet, powders and atropine. She excreted only 1.3 per cent. of the test dose devised by Borson (p. 241). Vitamin B₁ was given in a single dose of 5 mg. daily by mouth and within ten days she was having one formed stool daily instead of three to five liquid ones. The dose was then reduced to 1 mg. twice a day. An interesting feature was recovery of the ability to take milk which had formerly caused digestive upsets.

Field, Robinson and Melnick [570] have found that patients receiving intensive alkali therapy for peptic ulcer and those with achlorhydria have subnormal excretions of vitamin B₁. *In vitro* experiments showed that as much as fifty-six per cent. of vitamin B₁ is destroyed when incubated with bile or pancreatic juice in the absence of acid gastric juice. Patients suffering from peptic ulcer or achlorhydria may therefore develop a vitamin B₁ deficiency unless they take in more of the vitamin than will protect a normal individual. A chronic varying vitamin B₁ deficiency over many years may explain the cord changes in pernicious anaemia.

Many claims have been made that vitamin B₁ is of value in the treatment of gastro-intestinal hypotonia. It has certainly been observed that the administration of foodstuffs rich in vitamin B₁ (e.g., wheat germ [821]) is helpful in overcoming atonic constipation, but the effect of the bran and fibre in these foodstuffs cannot be overlooked. The laxative action of yeast is also well known. There are many reports of success in the treatment of constipation by means of pure vitamin B₁ or a concentrate, but the difficulty of evaluating these reports is considerable, especially where defæcation with some individuals is almost a conditioned reflex. Tuohy [828], Borsook [182], Vorhaus [322] and his co-workers state that chronic gastro-intestinal disturbances such as constipation, dyspepsia, and abdominal discomfort are relieved by the administration of 10 mg. of vitamin B₁. Borsook treated sixty-seven patients, and recorded that eighteen improved on a diet rich in vitamin B₁, and thirty-seven of the remaining forty-nine responded to the pure vitamin.

Careful X-ray studies have been made by Wood, Splatt and Maxwell [642] on the effect of pure vitamin B₁ on gastric secretion and motility in man. They state that the administration of vitamin B₁ in doses of 8 to 10 mg. intramuscularly has no effect on gastric secretion, although it hastens the emptying time in those persons whose gastric emptying time is habitually much longer than normal. Vitamin B₁ does not influence the rate of evacuation of the stomach of those whose emptying time is

normal or rapid. Controlled studies on the supposed laxative action of vitamin B₁ have also been made in the rhesus monkey, the only animal suitable for measuring the effectiveness of cathartic drugs, by Loewe and Knox [648]. They found that in doses of 1 to 100 mg. per kilo orally for periods of two to seventeen days it did not increase significantly the laxative effect of phenolphthalein. The increase was eighteen per cent. with a variation of ± 17 . While a deficiency of vitamin B₁ may lead to an atonic condition of the bowels there is little evidence that vitamin B₁ has a laxative effect in adequately nourished persons. It is possible that the clinical material for the observations recorded above was selected from poorly nourished hospital patients.

So-called gastro-intestinal diets are traditionally over-supplied with starches and sugars, and with insufficient foods containing vitamins B₁ and C. Functional disorders of the gastro-intestinal tract are frequently related to insufficient supplies of the vitamin B complex [602]. In such circumstances restriction to certain of the therapeutic diets will have an additive effect and may precipitate deficiency disease. Stepp [603] and Hartsock [604] describe a gastro-intestinal syndrome in which vitamin B₁ deficiency may be a factor; the symptoms include: anorexia, loss of gastric acidity, gastro-intestinal atony, constipation and a marked tendency towards the development of intestinal inflammatory processes. Vitamin B₁ in doses of 5 mg. parenterally is said to have a definite effect in increasing the secretion of gastric hydrochloric acid when given in both normal and pathological states [576]. Where anorexia is associated with vitamin B₁ deficiency the administration of the vitamin restores appetite [605]. Schlutz, Knott [183], Ross and Summerfeldt [180] in controlled experiments on large numbers of children found that those receiving extra vitamin B₁ gain in weight more rapidly and have better appetites than the controls.

It is believed that an insufficient intake of vitamin B₁ may be responsible for the gastro-intestinal crises in children of school age and babies. Wilkins [824] records striking improvement in some cases among children after giving small doses of vitamin B₁. Partial vitamin B₁ deficiency was also described by Clements [645] in at least eight per cent. of 150 infants breast-fed up to six months. The symptoms attributed to vitamin B₁ deficiency were failure to gain weight at the normal rate, constipation and vomiting. The administration of vitamin B₁ to the child, or to the mother if still nursing cured these symptoms. It should be noted that in these cases of Wilkins and Clements a claim is made for vitamin B₁ therapy only in the presence of a definite deficiency of the vitamin.

Kollmann [651] administered vitamin B₁ as a diuretic in eighteen infants under eleven months, all of whom had severe forms of acute or chronic nutritional disturbances in the course of which œdema appeared. Injections of vitamin B₁ caused the œdema to disappear rapidly, and resulted in improvement of appetite and general condition. It is not clear whether the infants were suffering from a deficiency of vitamin B₁ or not.

In the treatment of gastro-intestinal conditions, *e.g.*, peptic ulcer, by means of special diets, and after operations on the gastro-intestinal tract, supplements of vitamins B₁ and C should always be given; from 2 to

5 mg. of vitamin B₁ and not less than 100 mg. of vitamin C per day should be administered.

Vitamin B₁ in Pregnancy. Reference has already been made to the increased need of vitamin B₁ in pregnancy. Some writers believe vitamin B₁ deficiency to be an aetiological factor in such disturbances of pregnancy as hyperemesis gravidarum pregnancy neuritis, cardiovascular disorders and the toxæmias of pregnancy. While vitamin B₁ deficiency can cause neuritis and cardiovascular disturbances, which are amenable to treatment with vitamin B₁, it is more likely that hyperemesis gravidarum and pregnancy toxæmia cause vitamin B₁ deficiency; the former by repeated vomiting and loss of gastric hydrochloric acid needed for the absorption of vitamin B₁ (p. 174), the latter by hepatic dysfunction interfering with efficient utilization of vitamin B₁ (p. 210). Wernicke's encephalopathy (p. 216), which has been attributed to vitamin B₁ deficiency, is a terminal phase in hyperemesis gravidarum [734, 735].

Nixon [734] refers to the triad—œdema, toxæmia and avitaminosis B₁—frequently seen in pregnant women in Hong Kong. The report of the University Clinic there for 1940 shows an alarming increase in the number of cases of eclampsia and beriberi; the more severe the eclampsia the higher the incidence of avitaminosis B₁, with concomitant increased mortality. Forty-five per cent. of the cases of eclampsia were complicated by vitamin B₁ deficiency. From a study of eight cases in this country Nixon showed that the excretion of vitamin B₁ was below that of normal controls, and the vitamin B₁ content of the placenta of eclamptic patients was also significantly low. Nixon's cases were seen in Hong Kong, where there is undoubtedly widespread beriberi amongst pregnant women which ranks high there as a cause of maternal death, being responsible for thirty-five per cent. of the cases. There is conceivably a relation between pregnancy toxæmia and frank beriberi. In the latter, congestion of the liver, with subsequent diminution in liver function, may be a contributory factor in the precipitation of pregnancy toxæmia.

Most of the writers associating vitamin B₁ deficiency with the toxæmias of pregnancy diagnose the former from a low urinary excretion of vitamin B₁. The fallacy of this has been pointed out before (p. 289). Other methods of detecting vitamin B₁ deficiency, *e.g.*, blood pyruvic acid, showed normal values in pregnancy toxæmia (Neuweiler [171]). Rose and his co-workers [560] failed to note any decrease in the toxæmia during the latter part of pregnancy in a controlled group of patients receiving 3 mg. vitamin B₁ and other members of the B complex daily. Horwitz and Farley [566] concluded from blood tests that thirteen of a group of 100 pregnant women were suffering from vitamin B₁ deficiency, and that ten of these developed neuritis, severe hyperemesis and six anorexia. The two latter conditions are likely to have caused a conditioned vitamin B₁ deficiency. Williams and Fralin [733] in a nutrition survey of over 500 pregnant women noted that eighty-four per cent. of a group showing excessive nausea and vomiting in early pregnancy had a vitamin B₁ intake of less than 2 mg. daily. It is difficult to say whether the low vitamin B₁ intake was the cause of the nausea and vomiting. The intake of other essential nutrients was also probably low. We would like to re-emphasize

the view that vitamin deficiencies are never limited to lack of a single vitamin. Even a professor of dietetics could not select a diet which was low in vitamin B₁ and adequate in other essentials.

The use of vitamin B₁ in the treatment of toxæmia of pregnancy has been disappointing. Yasunami [559], working in Japan, states that he found vitamin B₁ effective in the treatment of the condition, particularly in preventing convulsions in pre-eclamptic patients. This has not been confirmed by workers in America and Europe. Siddall [825], although he claims that the toxæmias of pregnancy are associated with a vitamin B₁ deficiency, failed to observe any improvement in patients with pre-eclampsia after giving them daily injections of 1 to 7 mg. vitamin B₁ daily for ten days. Strauss [657], Browne [886], and Kapeller-Adler and Cartwright [887] failed to observe any significant improvement in blood pressure, œdema or albuminuria in patients with toxæmia of pregnancy treated with vitamin B₁ in doses from 9 to 25 mg. daily. Kapeller-Adler and Cartwright state that in severe cases vitamin B₁ actually intensified the signs and symptoms of pre-eclamptic toxæmia in the patients studied. They consider the use of vitamin B₁ contra-indicated in view of the fact that it inhibits histaminase, an enzyme that normally hydrolyses histamine in the body. Kapeller-Adler believes that in normal pregnancy most of the histamine formed in the body is destroyed by histaminase, but that in the toxæmias of pregnancy it escapes destruction.

Widenbauer [609], Spitzer [610], Bernstein [571], Lund [568] and Willis [899] claim to have effectively treated hyperemesis gravidarum with vitamin B₁. In some cases other preparations such as vitamin C, suprarenal cortex hormone, and vitamin B₆ were used. Spitzer used doses of 10 to 20 mg. of vitamin B₁. Willis and others [888] gave doses of 25 to 50 mg. daily parenterally up to a total dose of 800 mg. The results were stated to be satisfactory, but not as good as with vitamin B₆. Most of these observations were not controlled and their evaluation is, therefore, difficult. Hyperemesis causes vitamin B₁ deficiency (p. 209), but why the administration of large doses of vitamin B₁ should cure the former is difficult to understand. It may, of course, restore appetite and improve liver function by improved carbohydrate metabolism. One also wonders why the suprarenal cortex hormone was given to some of these patients. Success has been claimed for many forms of treatment of hyperemesis, but it is probable that the success is due to the psychological effect of the treatment, particularly if this involves the use of the hypodermic needle.

According to Bickel [294] cardiovascular disturbances, due in certain cases at least, to vitamin B₁ deficiency, may develop during pregnancy in women who were previously apparently normal. They are likely to appear in connection with pregnancy toxæmias. Daily injections of 50 mg. of vitamin B₁ were stated to produce a cure. Stähler [614, 615] describes similar cases treated with injections of 10 mg. daily.

Large doses of vitamin B₁ and the B vitamins are said to relieve heartburn in pregnancy [889]. As vitamin B₁ has no spasmolytic action and has no effect on gastric secretion and motility its use for this purpose does not seem to have any rational basis.

Vitamin B₁ in Metabolic Diseases. Diabetes. Observations on the relationship between vitamin B₁ and carbohydrate metabolism suggested that vitamin B₁ deficiency might be a factor in the development of diabetes, and that treatment with the vitamin might be of some value in the management of the disease. Reports concerning its efficacy in human diabetes, however, are contradictory. Some of the work done on animals and the treatment of diabetic neuritis have already been reviewed (pp. 167, 252). Vorhaus, Williams and Watermann [271] state that they obtained improvement following the administration of vitamin B₁ to diabetics, the best results being obtained in patients suffering from obesity, lack of appetite, polyneuritis and diminished metabolic rate. Sciclounoff [291] states that of thirty-five diabetics, twelve showed improvement of symptoms lasting for periods of several days up to some months after the administration of vitamin B₁ orally or parenterally. The rise in blood sugar following the injection of glucose was stated to be less marked after the administration of vitamin B₁. Sciclounoff also states that vitamin B₁ was a useful addition in the treatment of diabetic acidosis.

Dienst and his co-workers [616] stabilized a group of diabetics on insulin and then administered a preparation containing vitamin B₁. They claimed that in every patient carbohydrate tolerance was improved to the extent of requiring 10 to 20 units less insulin a day. Hypoglycemic reactions were also prevented. Sendrail and Marceillac [617] administered both vitamins B₁ and C to ten diabetics and recorded a fall in blood sugar, diminished glycosuria, and an increase in weight.

According to Mosonyi and Aszodi [618] the injection of 5 mg. vitamin B₁ into diabetics produces an initial hyperglycemia followed by a protracted fall in the blood sugar level. The initial hyperglycemia is abolished by the simultaneous administration of 300 mg. vitamin C. Seven patients with moderate diabetes were given 800 to 2,000 mg. vitamin C and 1 to 10 mg. vitamin B₁ a day, and daily blood and urine sugar values were determined. It was found that the maintenance dose of insulin could be reduced after a few days, and often the patient was able to keep the urine sugar free without insulin for several weeks.

Others have not been able to reproduce these results. Thus Lawrence and Oakley [108] of the Diabetic Department, King's College Hospital, treated a fairly large series of diabetics with vitamin B₁ and insulin but could not observe that the former had any effect, for better or worse, on the carbohydrate tolerance or insulin requirements. Smith and Mason [555] kept two patients with severe diabetes on vitamin B₁ deficient diets and a third was given injections of glucose with and without the addition of vitamin B₁, but they were unable to find that the vitamin B₁ had any influence on the intensity of diabetes or the sensitivity of diabetics to the action of insulin. Kaufman [670] found in a series of ten controlled cases that 8 mg. of vitamin B₁ daily, in addition to that received in the food, had no effect on the blood sugar of diabetics.

Trasoff and Bordin [741] noted an improvement in five diabetics out of fifteen from 2 to 10 mg. of vitamin B₁ daily, but careful analysis of the causes of the improvement showed that it could not be attributed to the vitamin B₁.

Owens [684] and his collaborators, while they grant that the administration of vitamin B₁ results in prompt improvement in diabetic neuritis, failed in well-controlled experiments to observe any reduction in insulin requirement as a result of administering large amounts of vitamin B₁ and riboflavin. They do believe, however, that vitamin B₁ deficiency may play a part in diabetic coma, in which the most severe damage falls on the brain, the heart muscle, and the kidneys, organs which are very susceptible to lack of vitamin B₁.

Experimental vitamin B₁ deficiency does not affect carbohydrate tolerance, as judged by glucose tolerance tests, in either normal or diabetic animals, unless the degree of deficiency is extreme [884].

A considerable degree of vitamin B₁ deficiency has been reported in young diabetics [985].

Hyperthyroidism. There is a striking resemblance between some of the features of hyperthyroidism and vitamin B₁ deficiency. Anorexia, diarrhoea, constipation, hypo- and a-chlorhydria, cardiac enlargement, tachycardia, palpitations, fatigue, impaired muscular strength, neuritis, disturbances of carbohydrate metabolism, and "neurastheric" symptoms occur in both conditions. The administration of vitamin B₁ and a diet rich in vitamin B₁ has, therefore, been recommended in the treatment of the patient with hyperthyroidism.

According to Cowgill [198] a good practical rule to follow in such cases is to administer 60 μ g vitamin B₁ per 100 calories of the estimated total daily metabolism. Rowlands and Wilkinson [357] state that the blood vitamin B₁ is lowered in hyperthyroidism. A number of clinical observations have been made on the effect of giving vitamin B₁ supplements to hyperthyroid patients. Means [620] and his associates state that the vitamin favourably influences the clinical course of hyperthyroidism. Jacobi and Pomp [621], however, are of the opinion that hyperthyroid patients treated with vitamins A and B₁ do no better than controls treated with rest and diet alone.

The use of vitamin B₁ in the pre-operative preparation of hyperthyroid cases is discussed by Frazer and Ravdin [121], who point out the possibility that a lack of vitamin B₁ may intensify the cardiovascular changes produced by thyrotoxicosis. The routine pre-operative preparation of fifty hyperthyroid patients was supplemented by the hypodermic injection of 10 mg. vitamin B₁ every other day, and by 10 grams of brewer's yeast daily by mouth, and the results obtained were compared with a control group of twenty-eight. It did not appear that the vitamin had any direct antithyrototoxic action, and there was no marked difference in the B.M.R., or in the severity of the post-operative reaction, but the vitamin treated group showed improvement over the controls in respect of the degree of reduction in the pulse rate, the increase in weight and appetite, and in the length of time necessary for adequate pre-operative preparation. The improvement was more pronounced in the more toxic patients.

Williams [296] has observed not infrequently the co-existence of hyperthyroidism, pellagra and ariboflavinosis (riboflavin deficiency). The tongue is sometimes fiery red and its papillæ atrophic; hyperexia may be

replaced by anorexia, and loss of weight becomes marked; achlorhydria, "dyspepsia," delayed emptying time of the stomach and diarrhoea are fairly common. Tachycardia, cardiac dilatation, generalized oedema, decrease in circulation time and vasomotor disturbances are also encountered. Decrease in strength and tolerance to exercise are almost always demonstrable. Williams and his co-workers [296] observed that the blood cocarboxylase was below normal levels and that the blood pyruvate and lactate were elevated in thirty-four of forty thyrotoxic patients. This was also observed by Davis and Bauer [986]. Borson [414], using saturation tests, also found evidence of vitamin B₁ deficiency in patients with thyrotoxicosis. Williams, however, considering as a group his thyrotoxic patients, found no correlation between the amount of vitamin B₁ deficiency and the height of the basal metabolic rate. He prescribed brewer's yeast, 5 gm. daily, and vitamin B₁, 10 to 20 mg. daily over a period of four years for all thyrotoxic patients under his care. As a result they were found to experience a distinct subjective improvement: Williams considers that in thyrotoxicosis not only increased destruction of vitamin B₁ occurs, but that there is increased excretion in the sweat, faeces and urine, since hyperhidrosis, diarrhoea and diuresis are common in this condition.

Experimental work on vitamin B₁ and the thyroid gland is described on p. 169.

Gout. Vorhaus and Kramer [624] have reported relief of the pain of acute gout by the administration of vitamin B₁. Kühnau [625] has recently observed that the blood nucleotides of a large number of persons suffering from gout are raised (5 to 10 mg. per 100 c.c.; normal 2 to 4 mg.) and Birch and Mapson [626] have also observed a similar rise in beriberi. Kühnau noted that vitamin B₁ had no effect on the level of the nucleotides in normal blood, but that it caused a drop in the nucleotides and uric acid of the blood from gouty patients. He concludes that the removal of uric acid occurs by phosphorylation, for which vitamin B₁ is indispensable and that in gout the formation of purines is so increased that the normal amount of vitamin B₁ present does not suffice for their removal. Kühnau states that in patients with gout the intravenous injection of from 10 to 20 mg. of vitamin B₁ is followed in most cases by a sudden disappearance of pain, swelling and redness. Nine cases of gout were treated by Callahan and Ingham [575] by means of 15-80 mg. vitamin B₁ daily as well as by cincophen, balneo-therapy and a purine-free diet. They state that the period of disability was considerably reduced, but since other forms of treatment were employed besides injections of vitamin B₁, the exact rôle played by the latter is difficult to assess.

Dermatology. Several investigators have published premature and uncontrolled observations on the use of vitamin B₁ in such dermatological conditions as lichen planus, neurodermatoses, pruritus, alopecia and pyodermatoses. There is no rationale for its use in such conditions and the observations have not been confirmed. Madden [554] treated 112 patients suffering from psoriasis by various methods, including vitamin and hormone therapy, and with sulphanilamide, and bismuth selicylate. He concludes that the best method of treatment is a low fat diet, 8 mg. vitamin B₁ daily by mouth, and an exfoliating ointment. Of twenty-

seven cases treated with vitamin B₁ alone, nine showed improvement of involution, and fifty per cent. of the recurrences were effectively controlled by vitamin B₁ alone. Bigham [748] gives daily doses of 8 mg. of vitamin B₁ in conjunction with local therapy for the treatment of psoriasis.

Shock. Some interesting experimental observations have been made on vitamin B₁ and shock, which if confirmed may have some clinical applications. Govier and Greer [749] state that the average survival time of anæsthetized dogs, in which hæmorrhagic shock has been induced, is significantly greater in those animals treated with vitamin B₁ than in untreated animals. The dose given was 1-2 mg. per kilo followed by 0.5 mg. every two hours. The average survival time in the treated group was eight hours, in the untreated group three-and-a-half. In a second paper, Govier and Greer [749] report that the administration of vitamin B₁ lowered the level of keto-acids, lactic acid and sugar in the blood of the bled dogs. This work was repeated with rabbits by Maycock [584], who was unable to confirm it. More recently Govier [641] has studied the relationship between hæmorrhagic shock and the plasma vitamin B₁ level. He states that the resistance to shock is greater in animals with a raised plasma vitamin B₁; they withstand more bleeding—forty-five per cent. more than controls with a low plasma vitamin B₁—before developing severe hypotension, and they show a more rapid return to their normal blood pressure when hæmorrhage stops. Govier and Grieg [652] have shown that in dogs subjected to shock from hæmorrhage and in animals suffering from anoxic anoxia dephosphorylation of cocarboxylase occurs. If the bled dogs are given vitamin B₁ a resynthesis of cocarboxylase results. Govier believes that there is greater need for vitamin B₁ in hæmorrhagic shock because of the dephosphorylation of cocarboxylase; vitamin B₁ exerts its beneficial effect, according to him, by causing increased synthesis of cocarboxylase, although why the lack of this should increase shock is not clear. Alexander [915] has shown that the concentration of total and phosphorylated vitamin B₁ in the liver rises in prolonged hæmorrhagic shock. The non-phosphorylated vitamin B₁ of muscle also shows an increase, occurring at the expense of cocarboxylase.

This work opens up the possibility of using vitamin B₁ clinically in the treatment of shock, due to hæmorrhage, trauma, burns or wounds.

Irradiation Sickness. The administration of vitamin B₁ appears to afford some relief from the symptoms of irradiation sickness, which is characterized by nausea, vomiting, diarrhoea, nervous symptoms and headache, and which may occur after exposure to therapeutic doses of X-rays or the rays from radium. Martin and Moursund [654] prevent these symptoms by giving 6 mg. of vitamin B₁ orally and a high carbohydrate diet for at least two days before exposure commences. If vomiting occurs it is frequently relieved by an intramuscular injection of 6 mg. Imler and Wammock [656] and Sponheimer [655] state that injections of 10 mg. of vitamin B₁ daily give rapid and complete relief from the more severe symptoms of irradiation sickness in the majority of cases. In severe cases the dose is increased from 15 to 30 mg. daily by injection. Results following parenteral therapy are stated to be more effective than

those following the oral route. The headache, nervous and digestive symptoms are said to be relieved by this treatment, although according to Wallace [712], who employs 10 mg. of vitamin B₁ and 50 mg. of vitamin C parenterally, vitamin therapy does not control the diarrhoea. Johnston [658] obtained the best results with a combination of vitamin B₁ and nembutal. Whitmore [886], from observations on 122 cases, found that 6 to 9 mg. of vitamin B₁ daily prevented symptoms of irradiation sickness in 80 per cent. of the cases. The dose was increased if sickness developed. Another report from Bean, Spies and Vilter [940] favours the administration of 50 mg. of vitamin B₁ and 300 to 500 mg. of nicotinic acid daily. These workers state that the incidence of irradiation sickness is greater in patients consuming diets poor in the vitamin B complex. Once irradiation sickness was established, the administration of vitamin B₁ and nicotinic acid had little effect in relieving it, although it was found that if given in the doses stated before exposure, the onset of the sickness was largely prevented. Bean, Spies and Vilter suggest that the basic disorder in irradiation sickness is a disturbance in the respiratory enzyme systems, of which vitamin B₁ and nicotinic acid are components.

Resistance to Fatigue. There are several premature reports on the beneficial effects of giving vitamin B₁, either alone or with other vitamin mixtures to increase the capacity and resistance to fatigue of athletes, soldiers and men engaged in heavy work [387, 660, 700]. These observations, which were largely uncontrolled, have not been confirmed. Many critically controlled studies on the subject have been made in America and the conclusion drawn by four separate groups of investigators is that the administration of vitamin B₁ or members of the vitamin B complex to adequately nourished persons has no influence on work output, endurance in dynamic work, recovery of working capacity, or recovery from fatigue [338-340, 796, 797]. In one group investigated there was no change in the heart rate, oxygen consumption, respiratory quotient, urinary excretion of nitrogen and ketone bodies, and blood lactate, nitrogen, glucose and B.B.S. [797]. The Council on Foods and Nutrition in America [841] have pointed out the waste of material and money in the indiscriminate administration of vitamin mixtures to workers in industry with a view to increasing output and diminishing fatigue. As the Council points out, if the workers are adequately nourished additional vitamins serve no useful purpose; if they are not adequately nourished they need more or better food and not vitamins out of a bottle. There is ample evidence that on diets deficient in vitamin B₁ and the vitamin B complex there is a decreased work output in trained subjects, loss of ambition and efficiency, and poor recuperation [584, 697, 886, 890]. This subject is dealt with in further detail on p. 231.

These observations are in keeping with those on rats. Vitamin B₁ in large doses has no effect on the work performance of rats receiving adequate vitamin B₁ in their diet [912]. There is some evidence, however, that vitamin B₁ may exert a pharmacodynamic effect on isolated perfused muscle. The total work output of the gastrocnemius muscle of the frog is significantly increased by perfusion with fluids containing 0.01 milliequivalents of vitamin B₁ and calcium pantothenate per litre [948]. This con-

centration of vitamin B₁ is actually many times greater than that occurring in skeletal muscle and the effects may well be due to vasomotor changes.

Vitamin B₁ deficiency is not a significant factor in producing fatigue and other symptoms (effort intolerance, breathlessness, palpitation, præcordial pain and subjective feelings of fatigue) in patients with the effort syndrome [987].

Morphine Addiction. Fitzhugh [666] observed that the irritability of rats addicted to increasing doses of morphine was reduced by vitamin B₁. The vitamin also prevented the increase in irritability that follows morphine withdrawal. Himmelsbach [187] has been unable to confirm this clinically in the case of morphine addicts.

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CHAPTER IV

RIBOFLAVIN

(FORMERLY LACTOFLAVIN, VITAMIN B₂, VITAMIN G)

HISTORY

IN 1932 Warburg and Christian [1] described a new "yellow enzyme" which they obtained from aqueous extracts of bottom yeasts. They claimed that it played an important rôle in respiration by forming part of an oxidation-reduction system, acting as a carrier of molecular oxygen to an oxidizable substrate. Warburg and Christian [2] later separated this "yellow enzyme" into a protein component and a pigment component, and noted that neither alone was catalytically active. In 1933 reports appeared from three independent research groups suggesting a relationship if not identity between vitamin B₂ or G, a heat labile factor of the vitamin B complex, and the water-soluble yellow-green fluorescent pigments found in many animal and plant products such as yeast, liver and kidney. Kühn [4, 5] and his associates in an attempt to isolate "vitamin B₂" from eggs obtained a water-soluble yellow-green fluorescent pigment, which like "vitamin B₂" had growth-promoting properties. They observed the similarity in distribution of the two substances and called attention to their probable relationship to the yellow enzyme of Warburg. Since pigments with apparent different vitamin activities were obtained from various sources they were looked upon as different members of a chemical group to which the term "flavin" was applied. The various flavins were called lactoflavin, hepatoflavin, ovoflavin, etc., according to their origin. Subsequent research has shown that they are all identical. At the same time Booher [6] in America showed that the growth-promoting activity of whey, which is a function of its "vitamin B₂" content, runs parallel to the amount of yellow pigment present.

Subsequent studies confirmed the identity of vitamin B₂ with the yellow pigment. After the elucidation of its structure and synthesis, it was decided in 1937 by the Council on Pharmacy and Chemistry of the American Medical Association to call it riboflavin and to abandon the terms vitamin G and B₂ [7]. This has met with acceptance in England, but on the Continent the name lactoflavin is still used. The spelling was changed in 1942 in the Addendum to the British Pharmacopœa to riboflavine. The original American spelling is used in this work as it is in general use in English-speaking countries.

CHEMISTRY OF RIBOFLAVIN

Riboflavin was isolated from many natural sources by Kühn [5] and his collaborators and their work has been confirmed by a number of other investigators. Among the sources were egg yolk and white, milk, liver, kidney, urine, grasses, fish retina, barley malt and yeast. Four synthetic

flavins differing from, but related to riboflavin, and possessing some vitamin activity, have been reported [8].

Riboflavin crystallizes in yellowish brown needles (Fig. 58). The needles have no sharp melting point, but darken at 240° C. and melt at 275°–282° C. with decomposition. Although stated to be water soluble its solubility is very slight, being only 12 mg. per 100 c.c. at 27.5° C. It is quite insoluble in the fat solvents. It is very soluble in alkali solutions. The solubility in water is increased by adding urea or by the formation of a complex with boron, by means of which an 0.8 per cent. solution may be obtained. Riboflavin is stable in strongly acid solution, but is unstable in the presence of alkali, or when exposed to light or irradiation with ultra-

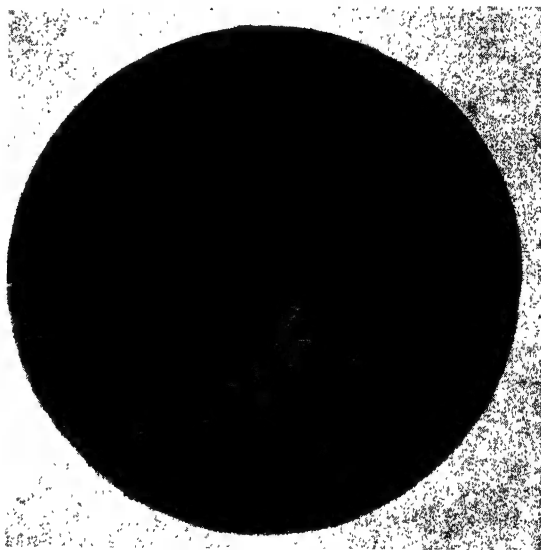
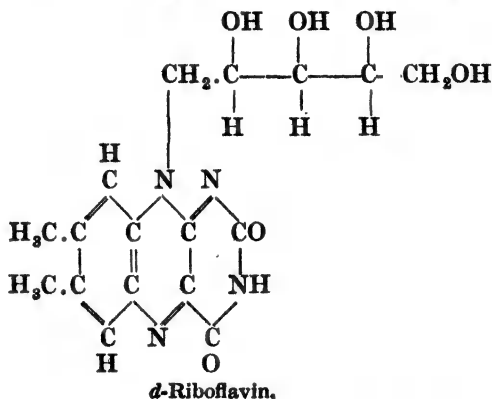


FIG. 54. Crystals of Riboflavin.

violet light, which cause irreversible decomposition. The vitamin is therefore stored in tubes covered with black paper or in amber-coloured ampoules. When exposed to daylight in neutral solution the ribose chain is split off to form lumichrome, which shows a greenish yellow fluorescence, but is devoid of vitamin activity. In alkaline solution light splits off the four terminal carbon atoms of the ribose to form lumiflavin, which shows a bright blue fluorescence and like lumichrome has no vitamin activity. Riboflavin is relatively highly thermostable (*e.g.*, only slight destruction at 120° C. for six hours) and uninfluenced by atmospheric oxygen in the dry state. Solutions of riboflavin exhibit a strong yellow-green fluorescence which reaches a maximum between pH 6.0 to 7.0. In milk at least ninety per cent. of the riboflavin appears to be in the free form. In most other sources, such as yeast, liver, and plants, it occurs conjugated with other compounds of high molecular weight.

Following the isolation of riboflavin in crystalline form, studies by Warburg and Christian [2] and by Kühn and Rudy [9, 10] in 1934, led to the elucidation of its constitution. The synthesis of riboflavin, which was essential for the final proof of its chemical constitution, was effected in 1935 by Kühn [11] and his co-workers and by Karrer [12, 13] and his collaborators. *d*-Riboflavin is 6:7-dimethyl-9-*d*-ribityl-isoalloxazine.



The yellow fluorescent pigment formed by the tubercle bacillus appears to be identical with riboflavin [116].

The fact that riboflavin exhibits a blue-green fluorescence in ultra-violet light serves as a basis for determining it by fluorometric methods [17-22, 145-148]. It is also estimated by converting it into lumiflavin by exposure to light in alkaline solution and then determining the lumiflavin colorimetrically. Biological methods are also still employed [14, 15]. A microbiological method has been developed which depends on observation that the growth of a specific strain of *Lactobacillus casei* and resulting production of acid is proportional to the amount of riboflavin in the medium [182, 188, 149-152].

UNITS OF RIBOFLAVIN

The Bourquin-Sherman [16] method of estimation, originally described as a method of assay for vitamin B₂ or G in the old nomenclature, has been shown to be only an approximate measure of riboflavin. The method is not specific since it is based on growth responses, and many other factors besides riboflavin are essential for growth. The Bourquin-Sherman unit was defined as "the amount of vitamin B₂ which when fed daily to a standard rat during eight weeks will cause an average gain of 8 gm. per week in addition to the average gain of the control test animals fed on a vitamin B₂ free diet." One Bourquin-Sherman unit has been variously reported as being equivalent to from 2 to 7 micrograms of pure riboflavin. The most recent figure is 2.19 micrograms [29]. There is no international unit.

Now that chemical and fluorometric methods are available for the estimation of the vitamin its concentration in a foodstuff or preparation

can be expressed in milligrams or micrograms per given volume or weight of material.

DISTRIBUTION OF RIBOFLAVIN IN FOODS

Riboflavin is widely distributed in plants, which synthesize it, and in animal tissues. Among the best sources are yeast, milk, white of egg, fish roe, kidney, liver, heart and growing leafy vegetables; other fairly good sources are fish, meat and poultry muscle. Grains and legumes, although they contain it, are not particularly rich sources. There is an increase in riboflavin during germination.

In green vegetables the leafy portions and growing parts contain most riboflavin; as the leaves get older and dry the riboflavin content diminishes. It has been shown that the milk from cows fed on fresh young grass contains more riboflavin than those fed on dried or root crops, which accounts for the fact that the riboflavin content of milk is highest in summer. Milk, eggs and leafy green vegetables are the chief sources of riboflavin in the average dietary. Liver and yeast are excellent sources, but since they are only consumed in relatively small quantities they do not contribute much riboflavin to the diet. Meat and poultry muscle are fairly good sources, but fish muscle is not. Fresh raw peas and beans are fair sources. White bread is a poor source. Extensive dietary surveys by Stiebeling and Phipard in America indicate that the riboflavin content of many diets is poor.

Effect of Cooking, Curing, Freezing and Canning. Since riboflavin is fairly heat stable, it is not appreciably destroyed in the ordinary processes of cooking, unless the medium is alkaline, although it is stated that the presence of cooking soda in a concentration of 0.12 per cent. causes no destruction [177]. The solubility in water will result in some loss in the cooking water if this is not consumed. The loss of riboflavin from food cooked by boiling is from fifteen to twenty per cent. [153]; slight losses also occur in pressure cooking [28]. With careful cooking up to ninety per cent. of the riboflavin present in the raw material may be retained [153]. Losses from roasting vary from fifteen to twenty-six per cent. [24, 153]. The losses are greater if the food is cooked while exposed to light, *e.g.*, up to forty-eight per cent. in the case of eggs, milk and chops [117, 154]. In the cafeteria cooking of food the loss of riboflavin is greater, from twenty-two to forty-five per cent. [155].

Considerable quantities of riboflavin in milk may be destroyed if milk bottles are allowed to stand for any length of time exposed to the sun or bright daylight [154, 809]. There is practically no loss of riboflavin in the pasteurization of milk [282]. In the curing of meat the losses of riboflavin are very small, *e.g.*, about eight per cent. The loss in frying is about the same as in roasting (twenty-three per cent.) [24]. Losses by drying or dehydration vary from nil up to fifteen per cent. [120, 156, 281]. Quick freezing has very little destructive effect on the riboflavin in foodstuffs [24, 25, 157]. Meat kept in cold storage for fifteen days loses fifteen per cent. of its riboflavin [24]. Canning and smoking have little effect on the riboflavin of foods, although in canning up to thirty per cent. or more may

be lost in the liquid used to can the food [26, 157, 281]. Appreciable amounts of riboflavin are retained in the preservation of food by brining and salting [280].

Riboflavin Content of Foodstuffs [158, 159, 283]

Food.	Description.	Micrograms of Riboflavin per 100 gram = $3\frac{1}{4}$ ozs.
<i>Cereals</i>		
Barley . . .	—	120-250
Maize . . .	—	63
Oats . . .	—	100
Rice . . .	Unpolished . . .	80
	Unmilled . . .	120
	Milled and polished . . .	47
Wheat . . .	Whole grain . . .	180-250
		Av. 280
	Germ . . .	480-1,500
	Bran . . .	600
	Whole grain bread . . .	180
	White bread (73%) . . .	50
	White flour (73%) . . .	85
	National flour (85%) (1943)	140-200 Av. 150
		(1945) 180
	National bread . . .	100-140
		(1943) Av. 100
<i>Proprietary Cereal Foods</i> [294]		
All-Bran . . .	Kellogg's . . .	360-480
Bran Flakes . . .	Post's (with added vita- mins).	210-290
Cerevim . . .	Lederle Labs. (with added vitamins).	3,300
Corn Flakes . . .	Kellogg's (with added vita- mins).	80
	Post's (with added vita- mins).	100
Force . . .	—	160
Grape Nuts . . .	With added vitamins . . .	170-200
Quick Quaker Oats . . .	—	140-180
Cream of Rice . . .	—	80
	With added vitamins . . .	620
Puffed Rice . . .	Quaker Oats Co. . .	60
Rice Krispies . . .	Kellogg's (with added vita- mins).	70
Shredded Wheat . . .	Kellogg's . . .	140-190
Soya Wheat . . .	—	270
<i>Fruits</i>		
Apple . . .	—	10-18
Apricot . . .	Dried . . .	100
	Tinned . . .	24

Food.	Description.	Milligrams of Riboflavin per 100 gram. = 3½ oss.
<i>Fruits—continued.</i>		
Avocado . . .	—	90
Banana . . .	—	56-75
Date . . .	—	45
Fig . . .	Fresh . . .	5
	Dried . . .	45
Grape . . .	—	15
Grape-fruit . . .	—	80
	Tinned . . .	19
Guava . . .	—	10
Orange . . .	—	15-47
	Tinned . . .	22
Peach . . .	Yellow . . .	60
	Tinned . . .	22
Pear . . .	—	20
	Tinned . . .	17
Pepper, green . . .	—	50
Pumpkin . . .	—	45
Pineapple . . .	—	5
Plum . . .	—	45
Prune . . .	Dried . . .	50
Pomegranate . . .	Juice . . .	100
Raisin . . .	—	29
Strawberry . . .	—	34
Squash . . .	—	50
Tangerine . . .	—	20
Water-melon . . .	—	15-60
<i>Nuts</i>		
Almond . . .	—	300
Coconut . . .	—	100
Pecan . . .	—	300
Peanut . . .	Raw . . .	500
	Roasted . . .	105
<i>Vegetables</i>		
Alfalfa . . .	Dried . . .	1,500
Arrowroot . . .	—	60
Artichoke . . .	—	30
Asparagus . . .	—	120
Bean . . .	Fresh . . .	110-175
	Dried . . .	Up to 750
Beet . . .	Tops . . .	300
	Root . . .	50
Broccoli . . .	Entire plant . . .	225
	Flower . . .	240
	Stem . . .	450
Cabbage . . .	—	50-57
Carrot . . .	—	50-60
Cauliflower . . .	—	105-180

Food.	Description.	Milligrams of Riboflavin per 100 gram = 3½ ozs.
<i>Vegetables—continued.</i>		
Celery . . .	—	100
Cucumber . . .	—	25
Endive . . .	—	200
Grass . . .	—	190
Kale . . .	—	400
Lentil . . .	Dried . . .	815
Lettuce . . .	—	100–150
Mango . . .	—	50
Mushrooms . . .	—	380
Onion . . .	—	24–50
Papaya . . .	—	150
Pea . . .	Fresh . . .	150–200
	Dried . . .	150–300
	Tinned . . .	20–50
Potato . . .	—	29–40
Radish . . .	—	80
Spinach . . .	—	230–400
Sweet potato . . .	—	40–75
Soya bean . . .	—	280–750
Turnip . . .	Root . . .	30–65
	Greens . . .	850
Water-cress . . .	—	270
<i>Dairy Products</i>		
Butter . . .	—	12
Cheese . . .	—	200–830
Eggs . . .	Whole . . .	250–440
	White . . .	280
	Yolk . . .	285
	Dried . . .	1550
Milk . . .	Cows', average (new) . . .	150
	„ range (new) . . .	185–210
	„ pasteurized . . .	119–206
	„ dried . . .	1,500
	„ „ (skimmed) . . .	160–200
	„ evaporated . . .	1,600
	Human . . .	16–52
<i>Meat Products</i>		
Calf . . .	Muscle . . .	140–220
	Kidney . . .	2,100
	Liver . . .	8,800
Chicken . . .	Muscle . . .	70–260
Lamb . . .	Heart . . .	270–850
	Kidney . . .	2,000
	Liver . . .	8,800
	Muscle . . .	250
Ox . . .	Heart . . .	900
	Kidney . . .	2,000

Food.	Description.	Milligrams of Riboflavin per 100 gram = 3½ ozs.
Meat Products—contd.		
Ox	Liver	8,000
	Muscle	225
	Brain	140
Pig	Bacon	180-800
	Muscle	150-200
	Ham	250
	Heart	900
	Kidney	2,100
Rabbit	Liver	2,700
	Liver	2,700-8,500
	Heart	550-1,880
	Kidney	1,810
	Muscle	860
Meat Extracts	—	1,540-2,580
Fish		
Cod	Muscle	45.8-810
	Roe	900-1,180
Conger Eel	Muscle	55
Crab	Flesh	350
Dogfish	Liver	870-540
	Roe	880
Haddock	Muscle	165
	Roe	1,420
Halibut	Muscle	185
Herring	Muscle	104
	Roe	385
Kippers	—	300
Lobster	Muscle	180
Mackerel	Muscle	660
	Roe	1,140
Oyster	—	180-420
Prawn	—	110
Sardines	Whole	410-620
	Tinned	110-180
Salmon	Whole	195-250
	Tinned	160
Trout	Roe	680
Turbot	Muscle	187
Miscellaneous		
Ale	—	390
Beer	—	50-170
"Bemax"	—	1,050
Chocolate	—	240
Coffee	—	170
Malt	—	560
"Marmite"	—	4,500-7,100 (Av. 6,000)

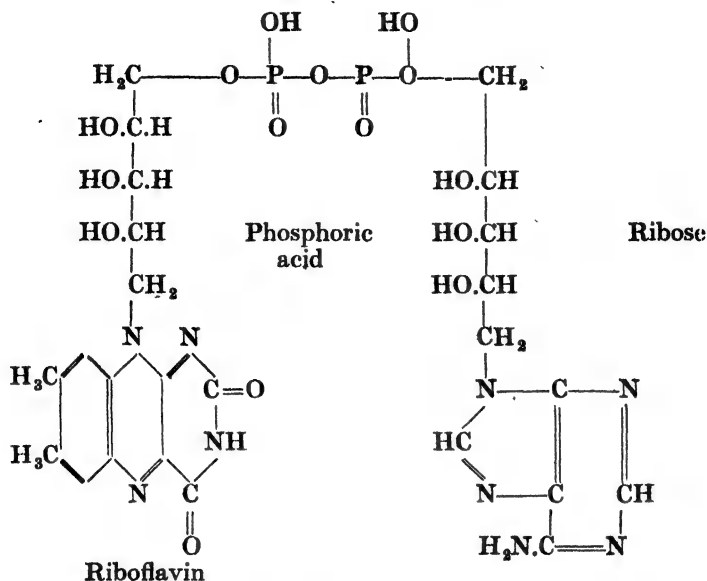
Food.	Description.	Micrograms of Riboflavin per 100 gram = 3½ ozs.
<i>Miscellaneous—contd.</i>		
Molasses . . .	—	62
Royal jelly . . .	—	820
Tea . . .	—	900
Yeast . . .	Bakers' . . .	2,500–4,000
	Brewers' . . .	1,800–3,000
	Dried . . .	1,243
	"D.C.L." . . .	5,000
	<i>Torulopsis utilis</i> . . .	5,000

PHYSIOLOGY OF RIBOFLAVIN

Riboflavin and Flavoprotein Enzyme Systems. Certain vitamins of the B complex, namely, vitamin B₁, adenylic acid, riboflavin, nicotinic acid, and possibly vitamin B₆, are of considerable importance in metabolism, since they form part of complex enzyme systems concerned in the oxidation reduction reactions of living cells. Such enzymes consist of a molecule of the vitamin, or a molecule of which the vitamin is part, coupled with a protein carrier, the whole exhibiting enzymic activity, or participating in a system which is necessary for the activation of an important enzyme.

In 1932 Warburg and Christian [1] isolated from yeast a yellow oxidation enzyme, which was subsequently shown by the researches of Kühn [81] and Theorell [82] to consist of an enzyme-like protein component closely associated with a flavin phosphoric acid, capable of undergoing reversible oxidation and reduction. Warburg [83] showed that the flavin was identical with riboflavin. Riboflavin phosphoric acid forms with a specific protein the "yellow oxidation enzyme" of Warburg, which was formerly thought to play an important part in tissue respiration. There is considerable doubt about the function of this enzyme in living tissues, although there is no question about the importance of other flavo-protein enzymes containing riboflavin such as *d*-amino acid oxidase [85], xanthine oxidase and succinic acid dehydrogenase [160, 161], and diaphorase [162].

The flavoprotein enzymes containing riboflavin are coenzymes linked to an apoenzyme, which is a specific protein. There are two different types of riboflavin coenzymes, namely, mononucleotides and dinucleotides. The mononucleotide is a riboflavin phosphate and the dinucleotide a riboflavin-adenine-dinucleotide. At least nine riboflavin coenzymes have been discovered, the properties of which are dependent on the protein apoenzyme with which the riboflavin containing prosthetic group is conjugated. Riboflavin-adenine-dinucleotide is :



The riboflavin-containing enzymes form part of a cycle for the transference of hydrogen. The various reactions catalysed by these enzymes are given in the following table :—

Enzyme.	Hydrogen Donor.	Hydrogen Acceptor.
Warburg's yellow enzyme [31-38].	Reduced codehydrogenases I and II.	Molecular oxygen.
Cytochrome <i>c</i> reductase.	Reduced codehydrogenase II.	Cytochrome <i>c</i> .
Diaphorase I	Reduced codehydrogenase I.	Cytochrome <i>a</i> and <i>b</i> .
Diaphorase II	Reduced codehydrogenase II.	?
Diaphorase [162]	Reduced codehydrogenases I and II.	?
<i>d</i> -Amino acid oxidase [85]	<i>d</i> -Amino acids	Oxygen.
Aldehyde oxidase [70]	Aldehydes	Oxygen-methylene blue.
Xanthine oxidase [163]	Xanthine	Oxygen.
Glucose oxidase	Glucose	?
Succinic dehydrogenase [160, 161]	Succinic acid	?
Fumaric acid oxidase [165]	Reduced dyes	Fumaric acid.

Warburg's yellow enzyme probably plays little part in cellular respiration because of its turnover number. This is the number of times per minute that the enzyme can accept hydrogen from the substrate and transport it to the next acceptor in the series. In one cycle the turnover number of Warburg's yellow enzyme is 50, compared with the figure of 8,000 for some other riboflavin enzymes.

Riboflavin enzymes or flavoproteins form part of the system for the metabolism of carbohydrate. This also involves codehydrogenases I and II, also known as coenzymes I and II, which are complexes of nicotinic acid amide, adenine, ribose and phosphoric acid (p. 381). The carbo-

hydrate substrate, *e.g.*, lactic acid, is oxidized by dehydrogenation through the nicotinic acid enzymes, codehydrogenases I and II, which are reduced to the dihydro compound. These in turn are oxidized by the riboflavin enzymes, which are at the same time reduced to the dihydro compound. The reduced riboflavin enzymes are then re-oxidized by specific reactions involving loss of hydrogen. Thus the hydrogen may react directly with oxygen or it may react indirectly through the cytochromes *a*, *b* or *c*. The following scheme has been suggested for the oxidation of lactic acid to pyruvic acid, before the oxidation of the latter to carbon dioxide and water [164].

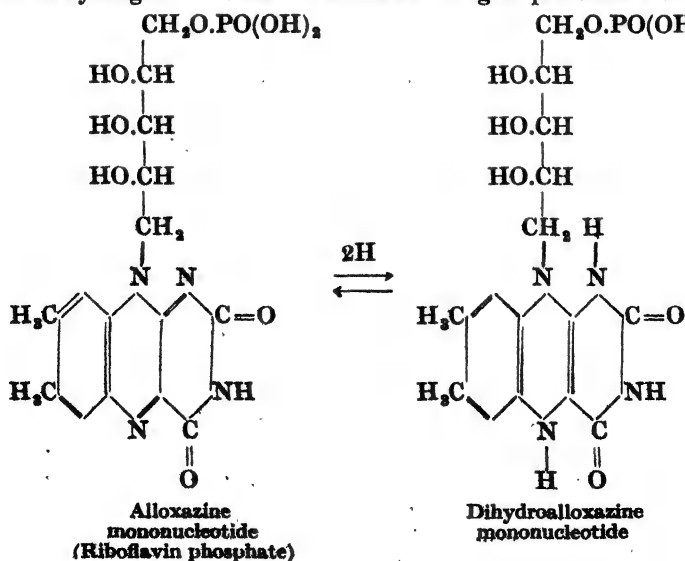
- I Lactic acid + lactic dehydrogenase + codehydrogenase I
→ pyruvic acid + reduced codehydrogenase I.
- II Reduced codehydrogenase I + flavoprotein
→ codehydrogenase I + reduced flavoprotein.
- III Reduced flavoprotein + cytochrome *b*
→ flavoprotein + reduced cytochrome *b*.

In the chain of the oxidative removal of lactic acid *vid* pyruvic acid to carbon dioxide and water it is probable that all the three vitamins, riboflavin, nicotinic acid and vitamin B₁, are essential, and that the absence of any one of them may interfere with the process.

The general scheme for the oxidation of a metabolite through the agency of one of the nicotinic acid coenzymes and flavoprotein is as follows :

1. Substrate + enzyme + coenzyme → Oxidised substrate + enzyme + reduced coenzyme.
2. Reduced coenzyme + flavoprotein → coenzyme + reduced flavoprotein.
3. Reduced flavoprotein + X → flavoprotein + reduced X.

X is a hydrogen acceptor, *e.g.*, cytochrome. The reduction and oxidation of the flavoprotein is supposed to occur by the addition and removal of hydrogen from the iso-alloxazine ring at positions 1 and 10 :



The fact that metabolically active tissues such as liver, kidney and heart muscle are rich in bound riboflavin, *i.e.*, flavoprotein, suggests that riboflavin plays the rôle of a respiratory catalyst [8]. It has also been shown that the riboflavin content of the liver, kidney and muscle is considerably lowered in animals receiving a diet deficient in riboflavin [166, 167]. This is also true of xanthine oxidase and *d*-amino-oxidase, which are both flavoprotein enzymes [168, 169]. According to Leeman and Pichler [170] the riboflavin content of various parts of the brain is directly proportional to the rate of respiration. A fall in the alloxazine adenine dinucleotide content of the muscle, liver and brain occurs when an animal is subjected to hæmorrhagic shock [805]. The brain is the first tissue to suffer. A fall in the cocarboxylase content also occurs (p. 271). It has therefore been suggested that riboflavin and vitamin B₁ should be given in cases of shock.

The need for riboflavin in the diet is probably due to the fact that it is an essential constituent of the flavoprotein enzyme system, which transports hydrogen from tissue cells to the blood stream, where free oxygen is available from the breakdown of oxyhæmoglobin, and where the oxidation of the hydrogen, received originally from the substrate, can be accomplished. It is possible that if hæmin compounds are not present in a tissue, the oxidation of metabolites is accomplished through the agency of the flavoprotein enzyme system.

Phosphorylation. Riboflavin must be phosphorylated before it can possess vitamin activity. This phosphorylation may occur as soon as it is absorbed from the intestinal wall, since preparations of intestinal mucosa can bring about the phosphorylation of riboflavin in presence of phosphates [86, 87]. It was originally thought by Laszt and Verzar [61-68], that the adrenal cortex hormone was essential for phosphorylation, but later workers have shown that this view is incorrect, since phosphorylation is normal after adrenalectomy [108, 110]. The phosphorylation of riboflavin probably occurs in the liver as well [8, 98], and human blood cells can synthesize both the phosphate and dinucleotide from riboflavin *in vitro* and *in vivo* [178].

According to De Preux [8] the liver not only stores riboflavin but also phosphorylates it, through the agency of the reticulo-endothelial system, since blockage of the latter reduces the amount of riboflavin phosphorylated, while stimulating it raises the degree of phosphorylation. There is evidence that other tissues, including muscle, can synthesize riboflavin dinucleotide [179].

Biosynthesis of Riboflavin. It has been clearly established that most micro-organisms including those in the rumen of herbivorous animals can synthesize riboflavin [58, 106, 172]. Synthesis also occurs in the cæcum of the rat, but only under certain dietary conditions. Although riboflavin deficiency does undoubtedly occur in man, it has not always been reported in subjects receiving diets low in riboflavin. It has for example been reported in subjects on diets providing 1 to 1.28 mg. of riboflavin daily, while others have failed to observe it in subjects receiving less than 1 mg. daily (pp. 824, 825). The possibility that under certain conditions intestinal

micro-organisms can synthesize riboflavin in man has been considered to account for this discrepancy. Najjar, Holt and their colleagues [297] have recently shown that in man the urinary and faecal excretion of riboflavin may exceed the intake. The faecal excretion in twelve subjects kept under observation for twelve weeks varied from 200 to 600 micrograms daily, although the intake was only 60 to 90 micrograms a day. The most likely explanation of this is that biosynthesis of riboflavin by the intestinal flora occurs. The conditions for this in man have yet to be investigated. The work of Najjar, Holt and their colleagues must certainly be considered in calculating the human requirements of riboflavin (p. 826).

Riboflavin and the Eye. Riboflavin appears to play an important part in the nutrition of the eye. Conjunctivitis and keratitis occur in animals on riboflavin free diets within seven to eight weeks, followed by a dulness of the eyeball and finally, according to some observers, opacity of the lens [38]. These eye lesions are no doubt due to defective metabolism in the lens and cornea following lack of the respiratory enzyme flavoprotein. The normal epithelium of the cornea of the rat has a high oxygen uptake, which falls if the animal is put on a diet deficient in riboflavin [295]. This fall in the oxygen consumption is probably due to necrosis of the epithelial cells of the cornea. Evidence for the rôle of riboflavin deficiency in the production of cataract is very conflicting. Day and other observers [38, 39] have described its occurrence in several species deprived of riboflavin, and its arrest in eighty-nine per cent. of the animals treated by the administration of riboflavin in doses of $120\mu\text{g}$ twice weekly. Bourne and Pyke [65], however, could only induce cataract in twenty to thirty per cent. of their animals by depriving them of riboflavin, and György [66] observed no cataract in five hundred rats treated in this way. The problem was reinvestigated by Eckhardt and Johnson [67], who produced cataract in only two out of twenty-three rats kept on a diet poor in riboflavin and rich in galactose. The subsequent administration of riboflavin did not prevent the cataract from forming in the second eye. Baum and his co-workers [122] state that rats on diets completely devoid of riboflavin do not suffer from cataract; they only do so if minute amounts of riboflavin are present, although a normal intake is non-cataractogenic. Riboflavin does not arrest the progress of lens opacities in the human eye [68].

Wolbach and Bessey [40, 41] have shown that corneal vascularization is an early, specific and most reliable criterion of ariboflavinosis, or riboflavin deficiency, in rats. They considered that this vascularization is a response to the respiratory needs of the corneal epithelium, in which oxidation occurs through the mediation of flavoprotein. El-Sadr [46] states that all the ocular manifestations of ariboflavinosis, with the exception of cataract, disappear on administering riboflavin. The ocular signs of riboflavin deficiency in human beings are discussed on p. 887.

Riboflavin has been found in the retinae of many species. Adler and Euler [181] consider that it plays some part in a light sensitive reaction because of the fluorescence of free riboflavin. They state that free riboflavin occurs in the fish retina and that it is therefore possible that in fishes riboflavin acts as a photosensitizer by absorbing short-wave light

and transmitting it as light of longer wave length. In the mammalian retina, however, Pirie [180] was unable to demonstrate much free riboflavin; it is nearly all bound as riboflavin-adenine-dinucleotide, which is light stable. There is therefore no evidence that riboflavin acts as a photosensitizer in the mammalian retina. Heiman [176] believes that riboflavin is essential for the visual act and may be a factor in cone vision by functioning in the flavoprotein oxidation reduction system and by its power to intensify weak light. He accepts the unproven assumption that riboflavin acts as a photosensitizer in the retina. This, incidentally, is accepted by many writers as an established fact, although the only evidence for this is the work of Adler and Euler [181] on the frozen fish retina. According to Philpot and Pirie [250], practically all of the riboflavin in ocular tissues is present as the adenine dinucleotide, and therefore not affected by light. They find that there is very little riboflavin in the cornea ($0.2\mu\text{g}$ per gram), but much more in the lacrymal gland ($6.5\mu\text{g}$ per gram). They therefore suggest that the riboflavin of the cornea is derived from the lacrymal secretions by diffusion. There are other observations which suggest some relationship between the nutrition of the eye and riboflavin. Dimness of vision, impairment of visual acuity, photophobia, lacrimation, inability to see in a dim light, visual fatigue and corneal vascularization have been described as clinical manifestations of riboflavin deficiency (p. 337). Kimble and Gordon [44] have observed that individuals showing poor dark adaptation and a low vitamin A blood level, did not improve with vitamin A alone, but responded to the administration of both vitamin A and riboflavin. This observation may mean that riboflavin plays some part in the visual act; on the other hand it may play a part in the absorption and utilization of vitamin A. Pock-Steen [78] observed that in a hundred and nine patients with incipient sprue (leiodystonia) and frank sprue many suffered from eye symptoms, the principal one being reduced visual acuity in dim light. This "twilight blindness," or aknephascopia, was considerably relieved by riboflavin, but not by vitamin A.

Riboflavin and Hæmatopoiesis. Several reports in the literature suggest a possible relationship between riboflavin deficiency and anæmia. Thus Miller and Rhoads [43] were able to produce in dogs a syndrome similar to sprue, a disease characterized by a macrocytic anæmia, by feeding diets deficient in riboflavin. György and his co-workers [48] also noted that riboflavin causes a definite increase in hæmoglobin production above the basal level when fed to dogs in which anæmia was produced by a deficient diet. More recently Spector and his colleagues [182] have shown that dogs on a synthetic diet normal in all respects, but containing no riboflavin, develop a severe anæmia on slight bleeding. The dogs do not recover unless given riboflavin in a dosage of $80\mu\text{g}$ per kilo daily, which appears to be sufficient for adequate hæmoglobin production. The anæmia produced by a deficiency of riboflavin was of the microcytic hypochromic type; when small amounts of riboflavin were given to the dogs after slight bleeding the anæmia was normocytic and hypochromic. Riboflavin also appears to play a rôle in determining the size of new blood cells according to Spector. Since *d*-amino-acids are deaminated by an enzyme system containing riboflavin-adenine-dinucleotide it is possible

that riboflavin may be related to amino-acid metabolism. Spector suggests that riboflavin may be concerned in the metabolism and arrangement of the amino-acids of the protein of the hæmoglobin molecule. In man there is no clear cut evidence that riboflavin is essential for hæmatopoiesis. There is often an associated anæmia in human ariboflavinosis, but this responds to iron [289]. A deficiency of riboflavin reduces phagocytic activity [274].

Riboflavin and Tumour Formation. Rats fed butter yellow (*p*-dimethylaminoazo-benzene) or treated with it externally develop cancer of the liver. This is prevented by giving the rats riboflavin, which appears to have an anti-carcinogenetic effect [183]. This is of some interest as the susceptibility of natives in the Far East and South Africa to primary carcinoma of the liver may be due to a dietary deficiency. The native diet in areas where these primary liver carcinomata are found is deficient in the vitamin B complex and riboflavin. The concentration of riboflavin in tumour tissue is low in comparison with that of normal tissues [184]. This is in keeping with the view that cancerous tissues have a deficient aerobic oxidation system. The possible relationship between leukoplakia and cancer of the tongue and diet is discussed on p. 387.

Riboflavin Deficiency in Animals. In the dog riboflavin deficiency causes bradycardia, cardiac arrhythmia, yellow mottling of the liver, degenerative changes in the central nervous system, collapse, and finally coma [49, 50]. These symptoms, which occur very rapidly, can be prevented by the administration of pure riboflavin. Pathologically, the most striking lesions occur in the liver and central nervous system, both of which are profoundly affected by changes in carbohydrate metabolism.

In the rat deprived of riboflavin the weight remains stationary; the animal develops alopecia and an eczematous condition of the skin affecting specially the nostrils and the eyes, the rims of which become denuded of hair [128]; and there is dulness of the cornea, blepharitis and conjunctivitis, the eyelids being stuck together with a serous exudate [51]. There is some evidence that the resistance of the rat to infection (*e.g.*, typhus and leprosy) is lowered [52, 53, 185] and that fertility is seriously impaired [186]. Increasing the fat level in a ration low in riboflavin has a deleterious effect on the growth of young rats; the administration of adequate amounts of riboflavin completely corrects this deficiency [118]. These and other observations suggest some relationship between riboflavin and fat metabolism [124]. Riboflavin is also essential for the nutrition of the pig and the monkey. Acute deficiency in the latter produces anæmia and a freckled dermatitis on the face, extremities and groin [288].

Riboflavin and Porphyrin Metabolism. It has been suggested by Vanotti and others [64, 69] that riboflavin is associated with porphyrin metabolism. He has observed that in some cases of sprue there is an increased output of porphyrins in the fæces, and that the administration of riboflavin and liver extract decreases their excretion considerably. A pathological porphyrinuria also occurs in human experimental riboflavin deficiency; the condition clears up after the administration of riboflavin [71]. This association of riboflavin with porphyrin metabolism, which is an iron-free pigment derived from hæmoglobin, is not surprising since in

the absence of riboflavin the functions of the enzyme flavoprotein are probably taken over by the respiratory pigments containing iron. The porphyrins are derived from these. Schneider [72] has shown that in a respiratory system containing iron pigments, the addition of cyanide, which inhibits respiration, causes an increased production of porphyrin. This does not occur if riboflavin is present in the system.

Riboflavin Metabolism and Other Vitamins. Other vitamins appear to play some part in riboflavin metabolism. Thus the storage of riboflavin in the liver, which occurs in a normal animal after injecting the vitamin, is considerably reduced if it is depleted of vitamin B₁ [178]. The riboflavin content of the tissues also falls considerably, mainly because of rapid excretion and poor absorption, in animals suffering from vitamin B₁ deficiency [174]. It has been argued that these changes in riboflavin metabolism are observed only in the terminal stages of vitamin B₁ deficiency and appear to be unspecific [175]. According to Delachaux [80] the metabolism of riboflavin is linked with that of vitamin B₁, since administration of large quantities of the latter results in an increased excretion of riboflavin and *vice versa*.

Pantothenic acid appears to have a direct and specific function as part of the mechanism whereby riboflavin is stored in the liver after the ingestion of food [178].

Chemically Induced Riboflavin Deficiency. It has been previously mentioned that vitamin deficiency can be induced in animals experimentally by feeding compounds structurally related to the vitamin. Thus pantooyltaurine can produce the symptoms of pantothenic acid deficiency and biotin analogues those of biotin deficiency (pp. 120, 125). Similarly the symptoms of riboflavin deficiency in animals have been reported following the administration of 2 : 4 dinitro-7 : 8-dimethyl-10-ribityl-5 : 10 dihydrophenazine, which is a structural analogue of riboflavin [284].

Pharmacology of Riboflavin. The pharmacology of riboflavin has been studied by Unna and Greslin [185], who state that in rats 10 grams per kilogram and in dogs 2 grams per kilogram orally fail to produce any toxic effects. The low solubility of the vitamin prevents its absorption from the gastro-intestinal tract in amounts sufficient to produce toxic effects. Likewise the subcutaneous injection of doses of 5 grams per kilogram produces no toxic effects [81]. The sodium salt is more soluble and 300 mg. of this intraperitoneally is lethal in the rat. Death occurs within two to five days with signs of anuria due to renal blockage with crystalline concretions. The daily administration of 10 mg. of riboflavin to rats and 25 mg. per kilogram to dogs for a period of four months produces no sign of toxic manifestations. The metabolism, circulatory and respiratory systems, and isolated smooth muscle organs of the animals are unaffected.

Absorption, Storage and Excretion of Riboflavin. Riboflavin is absorbed from the intestine and gastric hydrochloric acid is probably needed for its absorption. Phosphorylation is thought to occur in the intestine, although it can take place in the liver, blood and tissues. Riboflavin is not readily phosphorylated or absorbed in patients with gastro-intestinal disease; it is only utilized in such patients if it is injected [184]. The

bulk of the riboflavin in the body is stored in the liver, heart and kidneys. Rats maintained on a low protein diet are unable to store the normal amount of riboflavin in the liver [287]. On the other hand, deficiency of vitamin B₁ causes increased storage of riboflavin in the liver [304]. If there is a considerable increase in intake there is only a slight increase in the amount stored in the liver; and even if the animal dies for want of riboflavin the quantity present in the liver, kidney and heart is only about a third of normal [54]. The body therefore appears to cling tenaciously to its stores of riboflavin. Human blood contains about 0.5 μ g per gram [111]. Destruction of riboflavin in the body occurs, but to what extent is unknown. If riboflavin is injected there is an immediate concentration in the liver [57, 178]. The riboflavin content of the latter also increases during digestion and assimilation, being mobilized from other tissues [178].

When given intravenously the bulk is excreted into the small intestine, particularly the duodenum, from which it is reabsorbed [186]. It is largely destroyed in the large intestine, and some destruction also occurs in its passage through the kidney.

Certain factors influence the excretion of riboflavin. Thus in man the administration of large doses of vitamin B₁, *e.g.*, 10 to 8 mg., over a period, increases the excretion of riboflavin in the urine, although such dosage does not produce clinical riboflavin deficiency [187]. On the other hand, chronic vitamin B₁ deficiency is said to increase urinary excretion considerably in rats [288]. The protein intake also influences riboflavin excretion [188]. An increased protein intake produces a diminished excretion and increased retention of riboflavin. The excretion of riboflavin in rats is increased in experimental hyperthyroidism [189] and in man the excretion falls after exercise [287].

Riboflavin is also excreted in the sweat. The estimates of the amount lost in this way are variously given as from 5 μ g to 120 μ g per litre and 10 μ g per hour [198–195]. Even accepting the highest figure of 120 μ g per litre, this only corresponds to three per cent. of a good intake, and is, therefore, a negligible loss. According to Sargent, Robinson and Johnson [278] there is no free riboflavin in sweat.

If the diet is adequate riboflavin is excreted in the urine in the form of uroflavin, a pigment almost identical with it in composition, properties and vitamin activity. There is a fairly general dependence of uroflavin excretion upon riboflavin intake [109].

Emmerie [55], who has studied the riboflavin excretion in man, estimates that the urinary output of flavin is from 819 to 1,250 μ g daily. Strong [111] puts it at from 500 to 800 μ g a day on a normal diet, and 50 to 150 μ g on an intake of 1 to 2 mg. a day. This agrees fairly closely with the figures of Feder, Lewis and Alden [302], who found that the daily excretion was 500 μ g to 1,000 μ g, with an average of 800 μ g, on a daily intake of 2 to 3 mg. According to Connors, Eckardt and Johnson [192] the average riboflavin excretion of a group of normal subjects was 1,082 μ g. Keys and co-workers [278] state that on an intake of 0.81 mg. per 1,000 calories daily, the average daily excretion is twelve per cent. of the daily intake. An increased intake is reflected in an increased excretion; when the intake is considerably decreased, there is an increased retention and decreased excretion.

Riboflavin deficiency cannot be detected by a single determination of a twenty-four-hour specimen of urine. It fluctuates with the food intake and with the particular articles of food, *e.g.*, liver. Actually it is possible by placing human subjects on a diet low in riboflavin to depress the excretion to zero without evidence of clinical riboflavin deficiency [126].

Axelrod, Spies and Elvehjem [109] have carried out "saturation tests" on human beings with riboflavin, employing intravenous injections of 200 to 400 μ g of riboflavin per kilo of body weight, but they were unable to detect any correlation between the amount of the test dose of riboflavin retained and the daily urinary riboflavin excretion. Sebrell and co-workers [119] state that in a condition of riboflavin depletion there is a close relationship between riboflavin intake and excretion. Axelrod, Spies and Elvehjem observed that injected riboflavin was rapidly excreted

Urinary Excretion of Riboflavin before and after Test Doses

Author.	Subjects.	Daily Dietary Intake of Riboflavin.	Initial 24 hours Urinary Excretion. Mg.	Test Dose.	Excretion after First Test Dose.
Emmerle [55, 56]	Normal . .	?	0.952 0.885	5.71 mg.	3.28 mg. 1.20 mg.
Sebrell, <i>et al.</i> [119]	Normal . .	2.54 mg. to 3.68 mg.	0.793-1.265	5 mg.	50-80%
	Ariboflavinosis .	0.5 mg. .	0.024-0.119	2 mg.	Excretion rose from 34 μ g to 1.48 mg. on 20th day.
Axelrod, <i>et al.</i> [109]	Ariboflavinosis .	0.3 mg. per 2,000 cal.	0.058-0.091	0.2 mg. to 0.4 mg. per kg. i.v.	30-40% excreted after 1 hour.
Najjar and Holt [126]	Normal . .	?	0.236-0.270	1 mg. i.v.	?
	Normal . .	?	0.3-0.68 in 4 hours.	1 mg. i.v.	32-72% retained.
	Ariboflavinosis .	Very low .	0.074-0.194 in 4 hours.	1 mg. i.v.	81-98% retained.
Swaminathan [190]	Normal . .	1.2 mg. to 1.5 mg.	0.32-0.36	1 mg. to 10 mg. orally.	80-85% excreted.
Swaminathan and Verma [191].	Ariboflavinosis .	0.4 mg. to 0.5 mg.	0.05-0.2	5 mg.	10 to 20% excreted.
Connors, <i>et al.</i> [192].	Normal . .	?	1.03	1 mg. i.v. 5 mg. i.m.	38.6% retained. 21.5% retained.
	Patients with rosacea keratitis.	?	0.62	1 mg. i.v. 5 mg. i.m.	21.3% retained. 47.5% retained.
Williams, <i>et al.</i> [200]	Ariboflavinosis .	0.76 mg. .	0.06 to 0.15	0.103 mg. to 0.348 mg. after 4 hours.	0.103 mg. to 0.348 mg. after 4 hours.
	Normal . .	1-3 mg. .	0.46 to 1.8	2 mg. s.c.	0.311-1.155 mg.

in the urine, from thirty to forty per cent. being excreted after a test dose of 200 μ g per kilogram of body weight. They were also able to produce an uncomplicated riboflavin deficiency in the dog, in which the degree of retention of a test dose of riboflavin was found to be a measure of the riboflavin deficiency [125].

Another saturation test to detect human riboflavin deficiency has been devised by Najjar and Holt [126]. They inject 1 mg. of riboflavin intravenously after an overnight fast and follow the urinary excretion for half-hourly and hourly periods over four hours following the injection. Normally a marked excretion occurs in the first half hour, and during the second and subsequent hours the excretion falls off rapidly, approaching that of the initial control period. From thirty-two to seventy-two per cent. of the dose is retained. In riboflavin deficiency the excretion is much less, from

eighty-one to ninety-three per cent. being retained in the four-hour test. As the weight of the individual also influences the excretion Najjar and Holt suggest a dose of 0.016 mg. per kg. rather than a flat dose of 1 mg. for all subjects.

Feder, Lewis and Alden [802] state that saturation tests give no more information on the level of riboflavin nutrition than do single riboflavin estimations on a fasting morning specimen of urine.

The results of test dosing with riboflavin obtained by various investigators are given in the table on p. 828.

HUMAN REQUIREMENTS OF RIBOFLAVIN

The requirements of riboflavin have been calculated from (a) the analysis of normal diets, (b) the intake of riboflavin by subjects suffering from ariboflavinosis, or riboflavin deficiency, (c) from studies with experimental diets, (d) from excretion studies on riboflavin. In 1937 Stiebeling [59] from an analysis of the riboflavin content of diets calculated the daily intake as follows :—

Boys under 6 years of age	}	1.0 mg.
Girls " 7 " " "						
Boys 7 to 10 years of age	}	1.2 mg.
Girls 8 " 18 " " "						
Other children and adults		1.82 mg.

In Stiebeling's original paper figures are given in Bourquin-Sherman units. These have been converted to milligrams using the factor 2.19 (p. 808). In the same year Rose [60] estimated that the requirements of the child up to the age of ten were 0.88 mg. daily, and the adult 0.44 mg. per 100 calories, or 1.1 mg. per 2,500 calories. Rose's original figures were also given in Bourquin-Sherman units.

Stiebeling and Phipard [104] made a survey of the diets of a number of American families and concluded that the daily intake of riboflavin of the adult varied from 1.3 mg. to 1.8 mg. Actually many persons with a low income probably receive no more than 0.7 mg. to 0.8 mg. of riboflavin daily in their diet [196, 275]. A recent analysis of good mixed diets consumed in America showed a daily intake of 1.4 mg. of riboflavin per 2,500 calories [197]. Winters and Leslie [275] put it higher than this, viz., ninety-five per cent. of the National Research Council's requirements (p. 326), or about 2 mg. daily.

Macrae and co-workers [290] did not observe any signs of riboflavin deficiency in R.A.F. personnel on daily intakes of from 1.5 to 2.6 mg. (average 1.9 mg.). They therefore consider that the daily requirements do not exceed 2 mg.

Jones and his co-workers [277] had a camp of over 10,000 men of mixed races under their control in North Africa and noted signs of riboflavin deficiency on diets containing between 1 mg. and 1.28 mg. of riboflavin daily. On a diet containing 1.6 mg. of riboflavin daily deficiency symptoms were not observed. It is not stated whether these quantities of riboflavin in the diet were ascertained by chemical analysis or by

calculation from food tables. The latter method can give rise to very large errors, as not only are the vitamin contents of foodstuffs very variable, but much is lost in processing and cooking.

Signs of riboflavin deficiency, which are described later, have been observed in subjects receiving diets containing 0.8 to 0.5 mg. of riboflavin daily [119, 198]. On the other hand, Boehrer, Stanford and Ryan [199] gave three volunteers diets containing only 0.47 mg. of riboflavin daily for several weeks without any ill effects. It is possible of course that the experiment was not carried on long enough for deficiency symptoms to appear.

Sebrell and his co-workers [119] administered diets poor in riboflavin to ten women for periods from five to over eight months until signs of riboflavin deficiency developed. During the depletion period the excretion of riboflavin fell and a close relationship was observed between riboflavin intake and excretion. Supplements of riboflavin were given until the urinary excretion began to rise. From these studies the daily riboflavin requirements were calculated to be 8 mg. daily or 0.04 mg. to 0.05 mg. per kilogram of body weight in the adult. Williams and his colleagues [200] failed to observe any signs of riboflavin deficiency in subjects kept just over ten months on a standard diet adequate in all respects but containing only 0.35 mg. of riboflavin per 1,000 calories, *i.e.*, approximately 0.9 mg. per 2,500 calories. There was, however, a progressive decrease in the excretion of a test dose of 2 mg. of sodium riboflavin, indicating a progressive depletion of the tissue stores of riboflavin. An intake of 0.8 mg. of riboflavin per 1,000 calories was not associated with a depletion of the tissue stores as judged by the results of a test dose. Williams, therefore, thinks that an intake of between 0.35 mg. and 0.8 mg., *i.e.*, about 0.5 mg. per 1,000 calories is an adequate daily intake of riboflavin. This corresponds to 1.0 to 1.25 mg. daily on a diet furnishing 2,500 calories.

Keys and his co-workers [273] observed that a group of six normal young men showed no deficiency symptoms or physiological or biochemical abnormalities on a diet providing only 0.81 mg. of riboflavin per 1,000 calories, although adequate in other respects. Assuming a calorie consumption of 3,000 daily for moderately active young men, this corresponds to a daily intake of approximately 0.9 mg. of riboflavin. The tests included work on a tread-mill, an anaerobic work test, glucose tolerance test, tests of muscle power and psychomotor tests. Blood studies and slit lamp examination of the eye showed no abnormality and there were no abnormal clinical findings suggestive of riboflavin deficiency (p. 327). The average urinary excretion was twelve per cent. of the intake.

From excretion studies Emmerie [55, 56] considers that the daily requirements of riboflavin are 2 to 8 mg. Strong [111], using similar methods, gives 1 to 2 mg. of riboflavin daily as the minimal human requirement, with 2 to 5 mg. as the optimum. It is doubtful if many individuals receive as much as 8 mg. daily on present day diets. Indeed, a survey of the diets of munition workers in England in 1948 showed that their riboflavin intake was only 1.1 to 1.3 mg. daily [207].

The daily riboflavin requirements were also calculated from excretion studies by Oldham and co-workers [291], who found the daily intake of

riboflavin that caused neither a progressive decrease nor increase in the excretion of riboflavin when graded amounts of the latter were administered to subjects on a basal diet. The excretion of twenty per cent. of a test dose of riboflavin in four hours was taken as evidence of a satisfactory intake of riboflavin. Using these criteria the riboflavin requirements of children of pre-school age (two to five years) were calculated to be 0.5 mg. per 1,000 calories, or 1.5 mg. a day assuming a daily consumption of 1,500 calories.

The following are the recommended daily allowances of riboflavin suggested by the Food and Nutrition Board of the National Research Council, U.S.A. [201] :—

	Daily Riboflavin Requirements in mg.
Man (70 kg.) :	
Moderately active.	2.7
Very active	3.3
Sedentary	2.2
Woman (50 kg.) :	
Moderately active.	2.2
Very active	2.7
Sedentary	1.8
Pregnancy (latter half)	2.5
Lactation	3.0
Children up to 12 years :	
Under 1 year	0.6
1- 3 years	0.9
4- 6 „	1.2
7- 9 „	1.5
10-12 „	1.8
Children over 12 years :	
Girls, 13-15 years	2.0
16-20 „	1.8
Boys, 13-15 „	2.4
16-20 „	3.0

These figures for the riboflavin requirements of man may need revision in the light of the recent work of Najar, Holt and their colleagues [297] on the human biosynthesis of riboflavin (p. 317). They have demonstrated that riboflavin is synthesized in quantity in the human intestine, and believe that it may not be a dietary essential under all conditions. To what extent the biosynthesis of riboflavin normally occurs in man is not known. As suggested in the case of vitamin B₁ (p. 186), it may be that bacterial synthesis of riboflavin in the human intestine only occurs in any quantity when the oral intake of riboflavin is very low.

It is assumed from animal studies that the daily requirement of riboflavin in man depends upon sex, age, degree of physical activity and calorie intake, and is increased in pregnancy, lactation and fever. Requirements are increased as a result of physical exercise [47] and a high fat diet [57]. Ingestion of large amounts of nicotinic acid by patients on a deficient diet is said to increase riboflavin requirement [115]. Mills

[202] has shown that the requirements of riboflavin are independent of environmental temperature (*cf.* vitamin B₁).

THE RIBOFLAVIN DEFICIENCY SYNDROME. ARIBOFLAVINOSIS. DISEASES ASSOCIATED WITH RIBOFLAVIN DEFICIENCY

Historical. On reading the recent literature it would appear that the syndrome of riboflavin deficiency was first described in 1988 by Sebrell and Butler [82]. This is not so. The essential features were described as far back as 1911 by Stannus [84], whose early work on the subject has not received sufficient recognition. He clearly described a group of symptoms including soreness of the tongue and lips, with a sodden excoriated condition at the angles of the mouth (which he termed angular stomatitis) and palpebral fissures, and a characteristic lesion at the free border of the prepuce, the vulva and anus, together with a dermatosis of the scrotum, often spreading to the skin of the adjacent thighs. Stannus described the smooth tongue devoid of papillæ and denuded of epithelium—the so-called magenta tongue of ariboflavinosis—some twenty-five years before riboflavin was discovered. He knew that these lesions were the result of dietary deficiency but beyond that he could not go as the vitamins had not then been differentiated. In 1915 Bahr [208] gave further descriptions of the tongue lesions he had seen in Ceylon. Shortly afterwards, in 1918, Scott [189] described a condition among Jamaican coolies that showed some of the symptoms described by Stannus and in addition a central neuritis, photophobia, indistinctness of vision, ulceration and discharge of the eyelids and a burning sensation. A few years later Goldberger and Tanner [245] also gave an account of these symptoms, which they produced experimentally in American prisons. They noted that the condition was cured by autoclaved yeast. In 1930 Fitzgerald Moore [246] confirmed the observations of previous workers and added retrobulbar neuritis as part of the syndrome (p. 840), which he stated was common in West Africa, West Indies and Malay, and was responsible for the blindness of thousands of natives. The dietary origin of the syndrome was confirmed by curing it with marmite, a source of the vitamin B complex. Moore [247] later showed that neither vitamin A nor nicotinic acid deficiency played any rôle in the ætiology of the syndrome. Lastly Landor and Pallister [248] completed the clinical picture by describing the neurological symptoms—pains, tingling and weakness of the legs, rombergism, exaggerated knee and ankle jerks, diminished sensation in the feet, and defects in the touch, pain and deep sense pathways. These were also curable by marmite or liver. In 1988 Sebrell and Butler [82] published their observations on induced riboflavin deficiency, or ariboflavinosis as they termed it, in man.

Incidence of Ariboflavinosis. Judging from the literature riboflavin deficiency is relatively rare in the British Isles. It has been described here by Wilson [112], Duckworth [188], Deeny [204] and Scarborough [205], but the number of cases recorded is few. In certain parts of America, particularly the Southern States, it is stated to be common. Thus Goldsmith [206] states that forty to sixty-seven per cent. of hospital patients in Louisiana show some evidence of riboflavin and nicotinic acid deficiency.

Jeghers [227] states that he has found riboflavin deficiency very common among patients attending the Boston City Hospital. Of two hundred and eighty-four patients seen in a Newfoundland hospital, one hundred and forty-eight showed symptoms of ariboflavinosis [238]. According to Farber and Miller [228] riboflavin and nicotinic acid deficiency is common in tuberculous patients. Lesions stated to be characteristic of riboflavin deficiency were observed in twenty-five per cent. of four hundred patients in an American sanatorium. Riboflavin deficiency is very common among the Chinese according to Hou [105], and from the literature it would seem

Clinical Manifestations attributed to Riboflavin Deficiency (Ariboflavinosis)

Lips.	Tongue.	Skin.		Eyes.	Neurological.
		Face.	Elsewhere.		
Cheilosis (Angular stomatitis). * Vertical fissuring. Crusting of lips. Stomatitis. Burning of lips. Redness and des- quamation of lips.	Glossitis. Magenta tongue. Flattened papillae and epithellum. Burning tongue. Fissured tongue. Dysphagia. Patchy oval des- quamation.	Seborrhoeic der- matitis on alae nasi, nasolabial folds, eyelids and ears. Shark skin erup- tion. Comedones. Acne rosacea.* Capillary dilatation. Flushing of face.	Vaginitis. Dry itching dermatitis of hands, scro- tum, vulva and anus.	Roughness of the eyelids. Lacrimation. Photophobia. Blurred vision. Poor vision. Visual fatigue. Phlyctenular conjunctivitis. Impairment of visual acuity. Burning of the eyes. Twilight blind- ness. Circumcorneal injection.* Corneal vascu- larisation.* Retrobulbar neuritis. Blepharospasm.	Paraesthesiae, particularly in legs. "Burning feet." Exaggerated knee and ankle jerks. Rombergism. Partial optic atrophy. "Cerebellar syndrome." Ataxia. Muscular weak- ness. Nystagmus. Vertigo. Tremor. Clonic contrac- tions. Dysidiado- kokineses. Dysmetria. Mental apathy.
		Miscellaneous.		Opaque corneal infiltrates. Nebulae. Corneal epithe- lial dystrophy. "Rosy eyes," Rubeosels. Mydriasis. Iritis. Pigmentation of the iris. Cataract.*	
		Achlorhydria.*			

* The significance of these lesions is discussed elsewhere.

to be common in India [85, 198, 235, 236] and among African natives [84, 148].

Jones and his co-workers [277] noted that in a camp of over 10,000 men of mixed races in North Africa, some 1,746 or seventeen per cent. showed signs of ariboflavinosis. The outstanding features were stomatitis and cheilosis; the lesions responded to riboflavin therapy in a selected group of cases.

Spies [107] and his colleagues have repeatedly examined over a period of two years four hundred and seventy-two children of parents with deficiency diseases. Special attention was paid to riboflavin deficiency. Cheilosis was observed in a hundred and thirteen cases, linear lesions or fissures of the lips in ninety-three and ocular manifestations in a hundred and sixty-seven. Many were apathetic and made poor progress at school.

They frequently complained of sore mouths and burning and itching eyes, particularly in spring and summer. Increased exercise and infections seemed to precipitate the lesions in borderline cases. There was invariably a response to the administration of riboflavin or substances rich in it, and unless therapy was continued the children relapsed. Dietary studies showed that the mothers of these children had subsisted on grossly inadequate diets during pregnancy and lactation, and also that the majority of the children only received about thirty-five per cent. of the estimated requirement of riboflavin in their diet.

Lesions of Lips. In 1938 Sebrell and Butler [82] repeated Goldberger and Tanner's feeding experiments and induced a deficiency syndrome in eighteen women, who were given a diet complete in all respects, except that it was deficient in the vitamin B complex. Daily supplements of vitamin B₁ were given to eliminate any deficiency symptoms of this vitamin. Within ninety-four to a hundred and thirty days ten of the women developed cheilosis* (lesions on the lips), which began as a pallor of the mucosa of the lips in the angles of the mouth, and was followed by maceration and bilateral transverse fissures (Fig. 57). The lesions, which remained moist and became covered with a honey-coloured crust (Fig. 55), were identical with those described by Stannus [84] under the name angular stomatitis and by earlier workers. The fissures extend 1 mm. to 8 mm. on to the mucous membrane of the mouth and up to 10 mm. on the skin. They are usually shallow, but may be 0.5 mm. deep. The lips became abnormally red along the line of closure and show a marked increase in vertical fissuring, due to superficial denudation of the mucosa (Figs. 55 and 56). Sebrell and Butler also described a fine, scaly greasy desquamation on a mildly erythematous base in the nasolabial folds, on the *alae nasæ*, in the vestibule of the nose and on the ears. This syndrome was termed ariboflavinosis by Sebrell and Butler; Stannus [240] prefers the term hypo-riboflavinosis. It is identical with the *pellagra sine pellagra* and the *formes frustes* of pellagra described by Stannus and others between 1911 and 1935. Further cases resembling those of Sebrell and Butler were described by Jolliffe and his co-workers [88, 89].

Sebrell and Butler treated four of the ten volunteers with 1 mg. to 2 mg. of synthetic riboflavin daily for three to ten days and then with doses corresponding to 0.025 mg. per kilo of body weight. This was later increased to 0.05 to 0.075 mg. per kilo [87]. All the lesions disappeared in from five to forty-seven days, but in controls treated with 100 mg. of nicotinic acid daily the cheilosis was definitely worse. It cleared up in the controls, however, when they were given 0.25 mg. of riboflavin per kilo of body weight. One woman had the typical skin lesions of pellagra and cheilosis. After thirty days' treatment with nicotinic acid the pellagrous lesions healed, but not the cheilosis, which became worse, although it rapidly yielded to riboflavin in a few days. Jolliffe's cases cleared up with daily doses of 5 mg. [89].

* There has been some confusion over the term cheilosis and angular stomatitis, which some writers use interchangeably. Cheilosis is a lesion of the vermillion of the lip, angular stomatitis the lesion at the muco-cutaneous junction at the corner of the mouth.

Sir Philip Manson-Bahr [79] has cured the cheilosis and angular stomatitis of pellagrins by means of 3 mg. riboflavin daily. Sydenstricker [86]

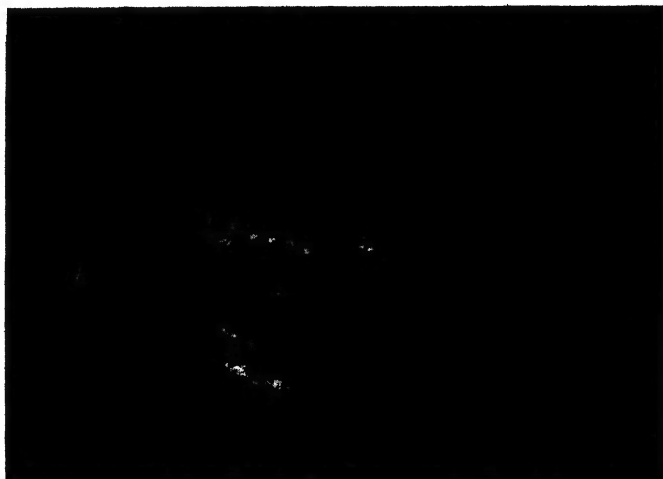


FIG. 55. Lips and Tongue in Ariboflavinosis. The case shows considerable erosion at the corners of the mouth (angular stomatitis). The lips are denuded, red and cracked and covered with crusts of blood. The tongue is fiery red and cracked.

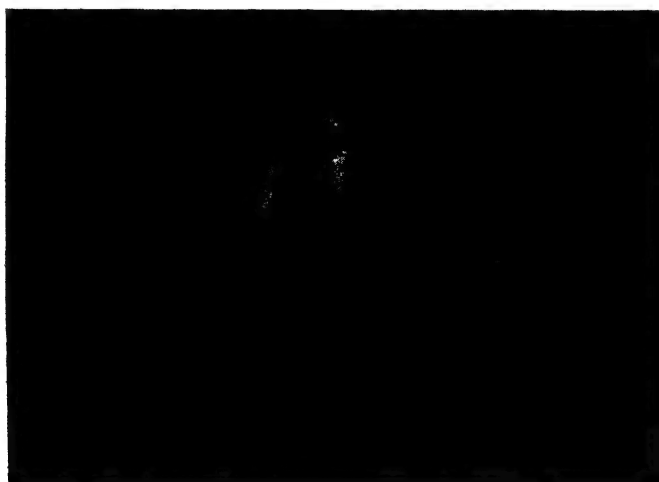


FIG. 56. Ariboflavinosis, showing Cheilosis. A case showing cheilosis with denuded lips and blood crusts. There is also considerable hyperplasia of the gums suggesting scurvy.

and his colleagues have also described five patients who showed evidences of pellagra or were pellagrins and presented lesions corresponding to those described by Sebrell and Butler. The cheilosis and fissures in the corners of

the mouth disappeared when riboflavin was given in rather large doses of 20 to 75 mg. a day orally, or 10 to 50 mg. parenterally; the riboflavin seemed more effective parenterally than orally. In every instance response to riboflavin was relatively slow, and in the presence of an inadequate diet nicotinic acid given concurrently seemed to have no adjuvant effect. Particular interest was aroused in two cases in which dermatitis, cheilosis and conjunctivitis appeared to be cured by riboflavin.

A case of ariboflavinosis with facial symptoms only has been described [292]. The condition cleared up with riboflavin therapy alone and reappeared when riboflavin was withdrawn. The patient had achlorhydria refractory to histamine, and defective absorption may have been the cause.

Although Kruse and co-workers [91] were able to duplicate Sebrell and Butler's results, some doubt has been cast upon the experimental production of pure riboflavin deficiency. Thus Boehrer, Stanford and Ryan [199] were unable to observe manifestations of riboflavin deficiency in volunteers on a daily riboflavin intake of 0.47 mg. The experiment only lasted for five weeks; possibly a longer period than this is necessary before symptoms appear. Williams and his collaborators [200] also failed to reproduce the oral and facial lesions described by Sebrell and Butler. They kept volunteers on diets providing 0.875 mg. of riboflavin daily for a period of ten months without observing any signs ascribed to riboflavin deficiency. Clinical examination of the volunteers showed no abnormal findings, and the following laboratory tests were normal: serum calcium, phosphorus and protein; blood lipids; blood counts; blood glucose, pyruvic acid; gastro-intestinal motility as shown radiologically; urinalysis; and slit lamp examination of the eyes. Machella and McDonald [208] doubt the existence of the ariboflavinosis syndrome. They have treated twenty patients with lesions attributed to the syndrome with riboflavin without success. It is possible that not only riboflavin deficiency, but deficiency of other factors of the vitamin B complex plays a part in producing the syndrome known as ariboflavinosis [240].

Validity of Angular Stomatitis* as a Manifestation of Riboflavin Deficiency. Angular stomatitis is not a specific sign of ariboflavinosis and may occur independently of the latter. Sutton and Sutton [209] stress the frequency of the condition in children and speculate on the possibility that angular stomatitis, or as they term it, *perlèche*, may be related to ariboflavinosis in a manner analogous to the fusospirochetosis of pellagrous stomatitis. Since fusospirochetosis may occur independently of nutritional deficiency it is possible that infected fissures of the lips and corners of the mouth may also occur without nutritional deficiency as a background. Spies [107, 210] has also shown that angular stomatitis commonly occurs in children and that *Staphylococcus aureus* and *Streptococcus haemolyticus* can frequently be cultured from the fissures. The condition often develops in children who dribble or constantly lick because of the abnormal amount of moisture at the corner of the mouth, whence the name *perlèche*, from *lecher*, to lick. One of the authors has also observed fissuring at the

* This is referred to as cheilosis in much of the literature cited. It is preferable to retain cheilosis for the lesions of the vermilion of the lips.

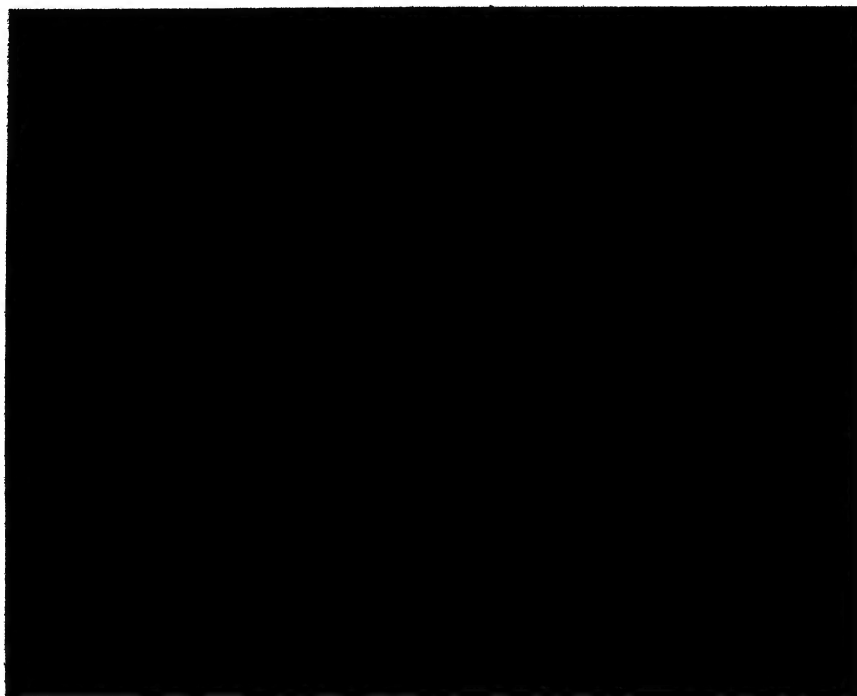


FIG. 57. Ariboflavinosis. The lids, particularly the lower, of both eyes are macerated and stuck together. There are also long wide fissures at the angles of the mouth (angular stomatitis).

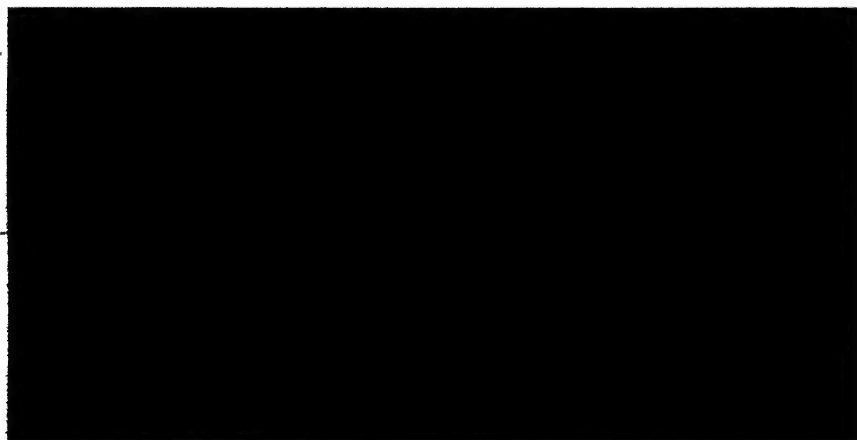


FIG. 58. Ariboflavinosis. The case shows dermatitis of both eyelids, extending from the margin of the lids to 3 to 6 mm. outwards with a dark red discoloration and papular lesions and crusts of exudates scattered over the lesion. The lesions responded to treatment with riboflavin and healed in six days.

corners of the mouth in two cases of Parkinsonism, in which there was no question of nutritional deficiency, and in which the condition was undoubtedly due to drooling of the saliva. Ellenberg and Pollack [211] have observed deep granulomatous fissures at the corners of the mouth with no involvement of the lips and glossodynia in thirty-four patients with no history of any nutritional defect and in whom laboratory studies showed no signs of avitaminosis. There was no response to intensive riboflavin therapy, either by mouth or parenterally. The cause of the lesion was eventually traced to badly fitting dentures causing mal-occlusion in thirty-two of the patients and mechanical defects in closure of the jaws in the remaining two patients. The skin at the corners of the mouth is constantly moist and becomes macerated and infected. Other writers have also observed angular stomatitis due to badly-fitting dentures and local trauma [212, 213], which did not respond to riboflavin, but disappeared when the dentures were changed. There is also evidence that angular stomatis may be associated with such diverse conditions as hypochromic anæmia [214], sensitivity to the constituents of chewing gum [215] and the dye in lipstick [218].

Numerous other reports show that the relationship of angular stomatitis to ariboflavinosis is not as clear cut as originally suggested and that as a symptom of the latter it is non-specific. Machella and McDonald [127, 208] failed to improve thirteen cases of angular stomatitis by treatment with riboflavin. Some responded, however, to nicotinic acid, vitamin B₆ and the entire vitamin B complex given as brewers' yeast. Smith and Martin [216] also noted that angular stomatitis may disappear after the administration of vitamin B₆. The observation of Machella [127] that hæmorrhagic lip lesions may respond to vitamin C suggests that some cases are scorbutic. Youmans [222] also found that some cases of angular stomatis were refractory to treatment with riboflavin.

Dermal Lesions of Ariboflavinosis. In the experimental production of ariboflavinosis Sebrell and Butler [82, 87] noted in addition to the labial changes a seborrhœic dermatitis of the face present on the *alæ nasi*, nasolabial folds, eyelids and ears. They were described in greater detail by Jolliffe [89], Spies [90], Sydenstricker [86] and others. One of the first descriptions of this seborrhœic dermatitis in pellagrins was given by Stannus [84]. The facial lesions consist of filiform excrescences of a seborrhœic nature, apparently derived from sebaceous glands, varying in length up to 1 mm., closely and sparsely scattered over the face. Although the characteristic location is in the nasolabial folds the excrescences occur on the *alæ nasi*, the bridge of the nose, above the eyebrows, about the ears and other parts of the body (Fig. 59). The lesion has the appearance of a seborrhœic dermatitis on an erythematous base; the skin over the excrescences is fine, scaly, greasy and desquamating. The skin over the nose has a "shark skin" appearance. The extruded sebum becomes inspissated in the pores with the development of fine hair-like protruding comedones resembling the urea frost of uræmia, only it cannot be rubbed off. It is prominent over the nose, malar eminences and forehead. Seborrhœic changes are stated to be present in the more severe cases of deficiency. In many cases there is also a crusty superficially

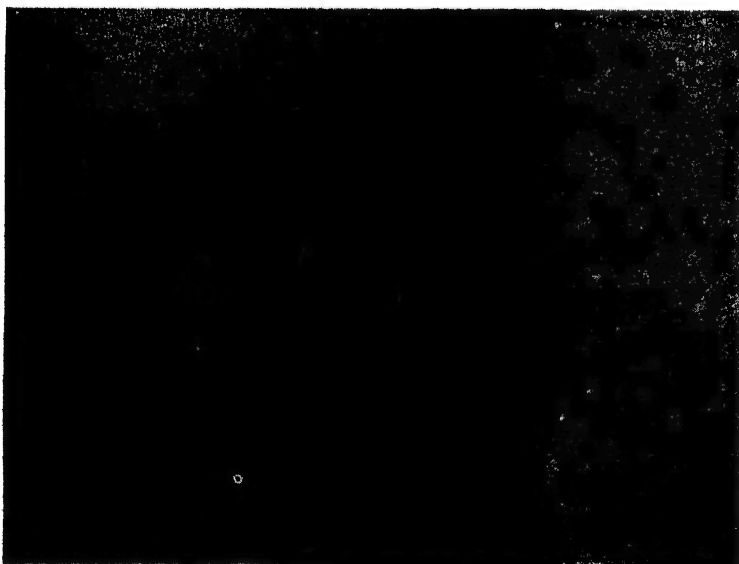


Fig. 59.—Ariboflavinosis before Treatment. A pellagrin showing filiform seborrheic excrescences on the forehead, nose, cheeks, lips and chin, and around the nasolabial folds. There are also moist pale patches in both corners of the mouth with vertical fissures on the lips.

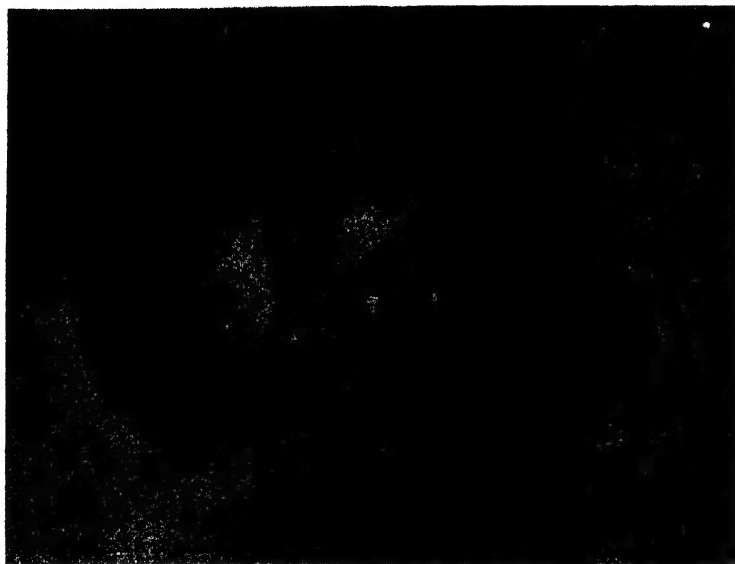


Fig. 60. Ariboflavinosis after Treatment with Riboflavin. The same patient as in Fig. 59 after treatment with riboflavin (15 mg. for the first two days, 10 mg. for the next seven to ten days, and then 5 mg. daily for another week). The lesions have disappeared.

eroded lesion just inside the nares, and there may be a vertical fissure at the mucocutaneous junction (Fig. 59). The eyelids often show a dermatitis and may be macerated and stuck together [105], as shown in Figs. 57 and 58.

Sydenstricker [217] also mentions a dry brown itching dermatitis of the hands and scrotum or vulva associated with cheilosis and glossitis and responding to riboflavin therapy. Purcell [148] describes a syndrome, which includes scrotal dermatitis and superficial glossitis and which disappears on treatment with riboflavin. Mitra [219] gives an account of an oro-genital syndrome among Indians, characterized by angular stomatitis, glossitis and scrotal dermatitis. The lesions did not respond to nicotinic acid, but did to yeast or 5 mg. of riboflavin daily. Some cases, however, appeared to do better on a mixture of riboflavin and nicotinic acid. As far back as 1911 Stannus [84] described a dermatosis of the free border of the prepuce, the vulva, anus and scrotum, spreading to the thighs, and he now thinks that this is part of the riboflavin deficiency syndrome [240].

Tongue Lesions of Ariboflavinosis. Jolliffe [89], Kruse [91] and their co-workers have described a specific type of glossitis associated with riboflavin deficiency and quite distinct from that attributed to nicotinic acid deficiency. The tongue in the latter may be sore before any changes are visible. Then desquamation of the epithelium of the lingual papillæ occurs, beginning at the tip and sides of the tongue and finally spreading over the entire dorsum. The tongue is scarlet, dry, atrophic and very painful. The tongue in ariboflavinosis on the other hand is described as magenta or purplish red in colour. It is clean and the epithelium does not desquamate over the papillæ, but is flattened and swollen. The papillæ are flattened or mushroom-shaped rather than atrophic, giving the tongue a pebbly or granular appearance. The tongue may be painful and burn when food is taken. Later irregular patchy denudation may occur giving rise to a condition known as "geographical tongue." Sydenstricker and his co-workers [115] have examined the papillæ microscopically and liken them to "a dead jelly fish that has been washed up on the beach . . . a round translucent hemisphere with the capillaries lying deeply in a loose coil." The scarlet tongue of pellagra or nicotinic acid deficiency owes its colour to desquamation of the epithelial cells of the papillæ; the capillary loops then become more readily visible. In the magenta tongue of ariboflavinosis the capillaries dilate and proliferate and possibly the circulation is slowed. This may account for the magenta colour of the tongue in contrast to the pink colour of the normal tongue.

Weisberger [220] describes a type of glossitis in riboflavin deficiency which does not correspond to the generally accepted picture. He states that the glossitis is characterized by a primary lingual coating followed by a patchy oval desquamation with an atrophic centre and raised edges. Weisberger states that this type of glossitis responds to treatment with riboflavin. Sydenstricker [115] noted a magenta-coloured glossitis in pellagrins on diets poor in riboflavin treated with nicotinic acid. The tongue returned to normal on exhibiting riboflavin.

Rosenblum and Jolliffe [221] record that some patients suffering from



FIG. 62. Ariboflavinosis in a Pellagrin. After treatment. The same patient as in Fig. 61 after treatment with riboflavin. The lesions have practically disappeared.



FIG. 61. Before treatment. The patient has ariboflavinosis. The lesions are inflammation of the skin, particularly on the fingers and palms. There are folds between the fingers.

vitamin deficiencies still had glossitis even when treated with both nicotinic acid and riboflavin, and that it did not return to normal until the whole vitamin B complex and vitamin B₆ were given. Machella and McDonald [208] failed to improve six patients with the so-called magenta tongue of ariboflavinosis by giving them riboflavin. Some did improve when yeast and vitamin B₆ were given.

When the tongue lesions of ariboflavinosis have been present for a long time the degenerative changes are not reversible and will not respond to riboflavin therapy.

The B Vitamins and Cancer of the Mouth. It is commonly taught that cancer of the oral cavity is preceded by degenerative changes in the oral mucous membrane which are recognized as being precancerous. Precancerous conditions in the tongue are leukoplakia, subacute or chronic inflammation, vascular injection, atrophy or hypertrophy of the papillæ and erosion of the epithelium. Most of these are stated to be produced by any form of chronic irritation such as tobacco, syphilis, dental trauma, sepsis and spirit drinking. Are the oral and pharyngeal symptoms of chronic riboflavin and nicotinic acid deficiency precancerous? Pain or burning of the tongue and degenerative changes in the papillæ of this organ are common in riboflavin and nicotinic acid deficiency, and the condition often progresses to scrotal tongue. Inflammatory hyperæmia occurs in the tongue in riboflavin deficiency. If these conditions become chronic do they become precancerous? Leukoplakia has been observed in patients suffering from vitamin B deficiency [224], but whether the connection is causative or coincidental is difficult to say unless more cases are studied.

Certainly the conditions are present in the chronic case of riboflavin or nicotinic acid deficiency for the development of leukoplakia. The Plummer-Vinson syndrome (œsophagitis, dysphagia, glossitis, anæmia) has been attributed to riboflavin deficiency [142, 225], and Ahlbohm [226] has established that it is a precancerous condition. Martin and Koop [224] believe that the intra-oral lesions of riboflavin and nicotinic acid deficiency are more common and of more importance than the other chronic irritants combined as precancerous factors, although they are unable to give statistical proof of this. They claim that there was a high incidence of vitamin B deficiency in their patients with oral cancer. In the treatment of the latter they give liver and yeast preparations as well as specific treatment for the lesion.

Ocular Manifestations of Ariboflavinosis. In 1939 Spies and his co-workers [90, 228] observed in patients suffering from malnutrition an ocular lesion, characterized by bulbar conjunctivitis, lacrimation, burning of the eyes and failing vision, that was cured by administering riboflavin. At the same time Sydenstricker and his colleagues [86] in a study on riboflavin deficiency noted that conjunctivitis and photophobia were prominent symptoms. In the same year Pock-Steen [45] described "twilight blindness" in patients with sprue or incipient sprue that was relieved by riboflavin and not by vitamin A. Many of these patients also suffered from ocular conditions such as reduced visual acuity, conjunctivitis, keratitis, and mydriasis, which were attributed to riboflavin deficiency [45, 78]. The work of Bessey and Wolbach [41] and Eckhardt

and Johnson [67] in 1939 showed that the earliest sign of riboflavin deficiency in the rat is corneal vascularization. This was followed in 1940 by the papers of Kruse, Sydenstricker and their colleagues [91, 92, 115] on the ocular changes of riboflavin deficiency in man. They emphasized that corneal vascularization was a constant finding. It is now known that this is not pathognomonic of riboflavin deficiency, although it may occur in cases of ariboflavinosis (p. 347). Sydenstricker [92] states that ocular manifestations precede all others in over fifty per cent. of cases of ariboflavinosis and eventually occur in about ninety per cent. The various ocular lesions of ariboflavinosis will be dealt with in detail.

Conjunctivitis. Gross injection of the vessels of the bulbar and fornix conjunctivæ have been described in subjects with riboflavin deficiency [91-98]. This has been referred to as "conjunctivitis," although no infection

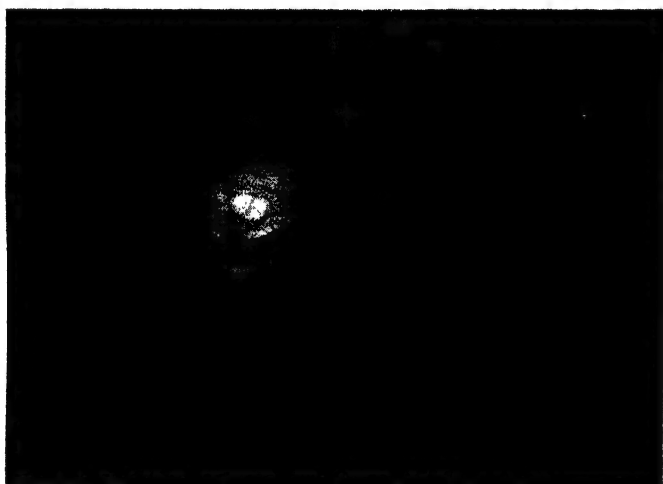


FIG. 63. Ariboflavinosis. A case showing injected vessels in the conjunctivæ, corneal opacities and phlyctenules at the margin of the cornea. Corneal vascularization was also present, but this is not pathognomonic (see p. 347).

was present (Fig. 63). Hou [105], who has seen many cases in China, adds phlyctenular conjunctivitis as a symptom of ariboflavinosis. He states that in China the ocular lesions are more commonly seen than the facial and oral ones.

It is stated that in India many patients with angular stomatitis and other signs of ariboflavinosis also suffer from angular conjunctivitis of the Morax-Axenfeld type [298]. This conjunctivitis, as well as the other signs of ariboflavinosis, is reported to have disappeared after treatment with 3 to 5 mg. daily, although sometimes doses of 40 mg. daily were needed.

Photophobia. Photophobia (Figs. 57, 58, 61) was observed in forty-three out of forty-seven patients with ariboflavinosis examined by Sydenstricker [92]. Johnson and Eckardt [98] and Hou [105] also found that it was a prominent symptom in their patients. Itching, burning, blepharospasm, a sensation of roughness of the eyelids, lacrimation, mydriasis,

blurred vision, inability to see in a dim light, and visual fatigue are also described as common in patients with ariboflavinosis [91, 92, 115]. These lesions clear up in twenty-four to forty-eight hours after giving riboflavin.

Diminished Visual Acuity and Eye Strain. Diminished visual acuity was observed in twenty-nine of forty-seven patients studied by Sydenstricker [92]. It was also noted in subjects with ariboflavinosis by Johnson and Eckhardt [98] and by Hou [105]. The symptoms occurred in the absence of errors of refraction or opacity of the lens. Impaired visual acuity may result from such symptoms as burning of the eyes, lacrimation, blepharospasm, corneal opacities and iritis, which are all stated to occur in ariboflavinosis. Vitamin A deficiency may cause some of these symptoms, and it is quite likely that a diet deficient in riboflavin—that is lacking an adequate quantity of milk, eggs, green vegetables and whole grain—is also deficient in vitamin A. The work of Kimble and Gordon [44] suggests that riboflavin may be necessary for the proper utilization of vitamin A. They found that some subjects with poor dark adaptation did not respond to treatment with vitamin A unless riboflavin was given as well. It is possible that the diminished visual acuity, photophobia and “twilight blindness” considered to be associated with riboflavin deficiency may be in part due to interference with the regeneration of visual purple in the rods and cones. Sydenstricker and others [92] report considerable improvement in visual acuity in their cases treated with riboflavin.

“Eye strain” has been stated to fall into this group. Pett [229] states that of a group of two hundred and thirty-two persons doing close work and with many complaining of eye strain, thirty-seven per cent. showed signs of riboflavin deficiency. Twenty-eight were given 3 mg. of riboflavin daily for three months and sixteen showed much improvement. However, a similar number in a control group showed improvement on a placebo. This illustrates the importance of suitable controls in studies of this kind.

Corneal Opacities. Bessey and Wolbach [41] in their studies on riboflavin deficiency in rats noted opaque infiltrates into the cornea from the edge of the limbus and Sydenstricker and his co-workers [92] observed corneal opacities in eighteen of his forty-seven cases of ariboflavinosis. Superficial nebulæ, seen by the naked eye as a slight “steamininess” and on slit-lamp examination as a fine superficial diffuse opacity, was noted in all these cases and superficial punctate opacities in two of them. Interstitial nebulæ and posterior punctate opacities were sometimes seen, although rarely. Johnson [180] noted corneal ulcers appear over these opaque infiltrates in severe cases of ariboflavinosis. These corneal opacities are probably caused by infiltration of the corneal epithelium and substantia propria with leucocytes. With riboflavin therapy these corneal nebulæ and ulcers heal, often completely, but if untreated the opacities become permanent and scar tissue may form from corneal ulcers. According to Sydenstricker [92] the interstitial nebulæ clear more rapidly than the superficial ones, and the posterior nebulæ disappear last of all. It must not be forgotten that a deficiency of other vitamins may result in corneal lesions. Thus vitamin A deficiency may cause corneal opacity, scarring of the cornea and even perforation in severe cases (p. 71), and corneal lesions are also seen in animals on diets deficient in pantothenic acid.

A deficiency of vitamin A and riboflavin may in fact occur in the same individual. Verghese [281] and Verma [282] describe a syndrome, which appears to combine the manifestations of a deficiency of both these vitamins, and which is characterized by partial degeneration of the optic nerve, phrynoderma, sore mouth, night blindness, xerosis, conjunctivitis, photophobia and impairment of vision. The condition responded to treatment with shark-liver oil, a potent source of vitamin A, and yeast, a source of the vitamin B complex including riboflavin. The oral lesions cleared up with riboflavin alone.

Cataract. Conjunctivitis and keratitis occur in animals on diets free from riboflavin followed by dulness of the eyeball and finally, according to some observers, opacity of the lens, although the latter observation has been doubted (p. 818). In Sydenstricker's series [92] cataract was observed in six, but they were all elderly patients and it is difficult to say whether the cataract was the result of riboflavin deficiency. According to Wagner, Richner and Karbacher [68] riboflavin does not arrest the progress of cataract in the human eye. A deficiency of other factors may cause cataract in the experimental animal, *e.g.*, tryptophane [280]. In man it cannot be considered as yet that cataract is a result of ariboflavinosis.

Iritis. Sydenstricker and his co-workers [91, 92] observed severe iritis in four out of forty-seven of their cases and mild iritis, characterized by moderate congestion of the iris with accumulation of pigment on its anterior surface, in somewhat under half of the cases. In light-coloured irises the pigment appeared as dark clumps of "hazel spots"; in brown irises the pigment caused "veiling of the normal architecture." As these changes disappeared with riboflavin therapy they were considered to be part of the ariboflavinosis syndrome.

Rubeosis Iridis. This is a peculiar non-inflammatory vascular proliferation affecting the iris and mostly seen in diabetics, although diabetes is not an essential factor in its causation. Festoons of newly-formed blood vessels are seen on the surface of the iris and in some cases the condition appears more particularly in the sphincter region where the vessels anastomose to form a network encircling the pupil. In other cases patches of anastomosing vessels are seen in the periphery. Stannus [288] suggests that rubeosis is a manifestation of riboflavin deficiency, as he has cured the condition with riboflavin in doses of 5 mg. daily. After forty-eight hours the vascular network on the iris is difficult to see, and after a week completely disappears.

Retrolbulbar Neuritis and Corneal Epithelial Dystrophy. Métivier [128] has given an account of ocular manifestations observed in Trinidad and which he considers are due to riboflavin deficiency. One he calls "tropical nutritional amblyopia," which is the nutritional "retrolbulbar neuritis" of Moore [246, 247], Landor and Pallister [248] and Scott [189]. There is failure of central visual acuity, diminution in the size of the visual fields, scotomata, pallor of the temporal halves of the discs, and partial optic atrophy. This is attributed to failure of the nutrition of the optic nerve, either directly or through the retinal elements, and according to Métivier is the result of ariboflavinosis, since recovery occurs on administration of 4 mg. to 5 mg. of riboflavin daily. Foods rich in the vitamin B complex,

such as wheat germ, yeast and marmite are also effective in causing improvement. Wilkinson and King [256, 301] have also described a deficiency syndrome seen in Hong Kong in 1940 with amblyopia as a predominant symptom accompanied by soreness of the tongue, angular stomatitis, giddiness, weakness of the limbs, temporal pallor of the discs, acroparæsthesiæ, scrotal eczema and swelling of the ankles. Visual acuity was reduced in some cases to finger counting at three feet within a few weeks of onset. The condition cleared up with yeast and dietetic measures and also with nicotinic acid and riboflavin, although the authors regarded the syndrome as a result of nicotinic acid deficiency. The amblyopia, however, cleared most rapidly when the patients were given riboflavin. Thus 3 mg. of riboflavin daily brought vision from $\frac{6}{60}$ to $\frac{6}{12}$ or $\frac{6}{18}$ in a week or ten days. It was also found that a full well-balanced diet helped to restore visual acuity. The complete syndrome is more likely to be due to a deficiency of riboflavin and protein (swelling of ankles) rather than of nicotinic acid.

Métivier claims that he has observed a hundred and ninety-two cases of a condition hitherto unrecorded which he calls essential corneal epithelial dystrophy. It is characterized by a faint greyish-white disturbance in the corneal epithelium made up of fine points like dots and commas, and it runs typically in a double line transversely across the cornea at the level of the lower part of the pupillary areas. It stains with fluorescein or Bengal red. At times the double line is incomplete, but minute prolongations usually extend above and below it; in some cases almost the whole of the corneal surface is covered with faint greyish-white points. Other symptoms associated with this eye condition are photophobia, lacrimation, pallor or atrophy of the temporal half of the optic disc, burning and numbness of the feet, cheilosis, dry parched skin, sore tongue and "rosy eyes." The last-mentioned is an apple-pink injection of the ocular conjunctiva exposed in the interpalpebral area with dilated vessels that run into the limbus. Recovery occurs from the epithelial dystrophy and rosy eyes after two weeks' treatment with 5 mg. of riboflavin daily, and only after five to twelve weeks when foods rich in the vitamin B complex are given. The burning and numbness of the feet was only relieved by vitamin B₁.

Snow-Blindness. According to Tisdall [287] snow-blindness may be due to riboflavin deficiency. He examined four hundred Indians in Hudson Bay and noted that five per cent. were blind. This blindness he attributes to riboflavin deficiency because the diet is poor in riboflavin and to the light reflected from the snow being very strong and causing local destruction of riboflavin in the eye. Pure riboflavin is destroyed by ultra-violet light, but in the eye it is present as riboflavin-adenine-dinucleotide, which is not inactivated by light [250]. Four cases of acute snow-blindness observed by McDevitt and others [239] in Newfoundland, were stated to respond to treatment with riboflavin.

Corneal Vascularization. In 1940 Sydenstricker, Sebrell, Cleckley and Kruse [91, 92] examined the eyes of forty-seven patients with riboflavin deficiency and noted vascular changes in forty-five of them. They state that the earliest and most common sign of ariboflavinosis is circumcorneal

injection (Fig. 65) that is, proliferation and engorgement of the bulbar conjunctival capillaries of the limbic plexus. The lesion, if not grossly visible, is seen on slit-lamp examination. They considered circumcorneal



FIG. 64. Normal Eye. There is no proliferation of the limbic vessels and no penetration of cornea by blood vessels.

injection as pathognomonic of ariboflavinosis. In cases not showing circumcorneal injection these authors observed gross injection of the vessels of the fornix and sclera. They describe the earliest change as marked proliferation and engorgement of the limbic plexus with the production of great numbers of narrow capillary loops, which outline the

extreme margins of the scleral digitations and obliterate the narrow zone between the plexus and the sclerocorneal junction. Sydenstricker states that if untreated the lesion progresses to corneal vascularization within



FIG. 65. An eye showing circumcorneal injection. The vessels of the limbic plexus are beginning to proliferate.

a short time, but rapidly regresses if treated with riboflavin. In untreated cases the cornea is invaded first by very small capillaries arising from apices of loops surrounding the scleral digitations and lying just beneath the epithelium. They soon anastomose to form a tier of loops from which more capillaries develop and extend centripetally to form secondary

arcade capillary loops. The process of anastomosis and loop formation proceeds until extensive vascularization of the cornea results (Fig. 66). The capillaries are empty at first but fill with red blood cells in a few

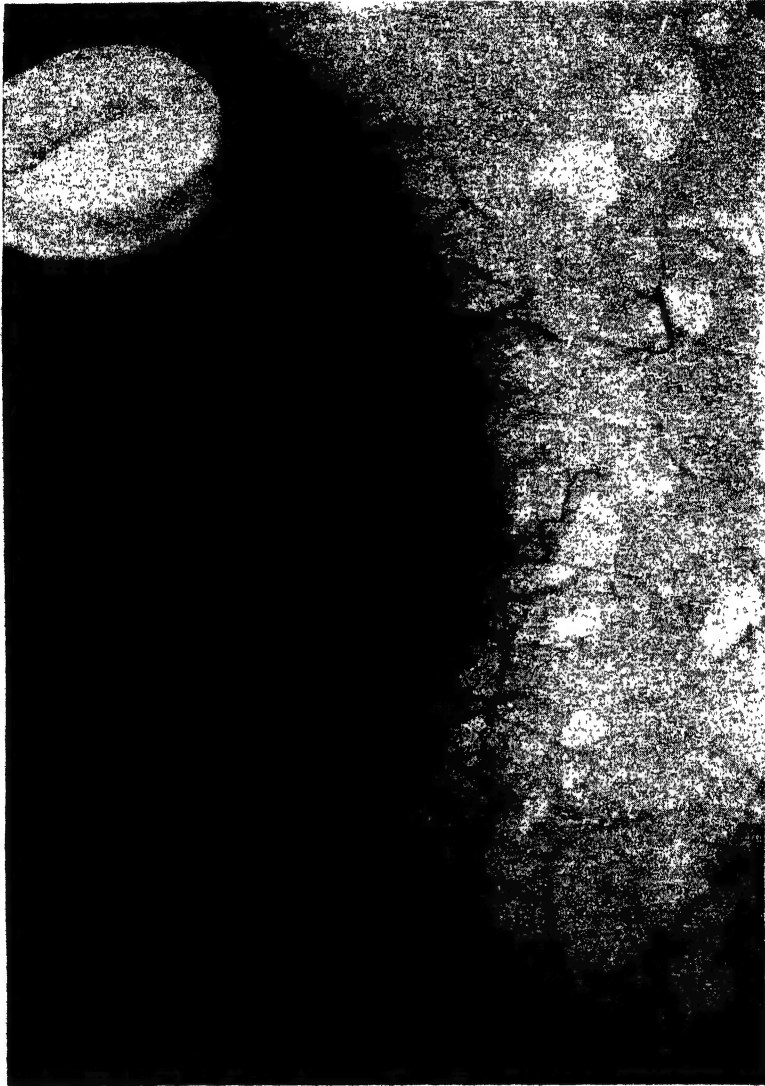


FIG. 66. An eye showing further proliferation of the vessels of the limbic plexus and penetration of the cornea with twigs, streamers and loops.

days. According to Sydenstricker the capillaries may penetrate the substantia propria and even reach the subendothelium if the deficiency is severe and prolonged. Many of the vessels are so small that they are invisible to the naked eye or with a loupe but are visible under the corneal microscope or slit-lamp. The superficial character of the keratitis is said

to distinguish it from syphilitic and tuberculous keratitis with their deep and posterior capillary infiltration. The Sydenstricker school believe that the eye lesions described are due to riboflavin deficiency because they clear



FIG. 67. The same eye as in Fig. 66 after treatment with riboflavin. There is some regression in the degree of corneal vascularization.

up when riboflavin is administered; they are present in persons living on diets poor in riboflavin; the administration of other vitamins has no effect; the lesions are associated with cheilosis and glossitis; and similar lesions are produced in animals kept on diets deficient in riboflavin.

Verma [235] and Aykroyd and Verma [236] have reported a high incidence of superficial keratitis associated with cheilosis, stomatitis, fissured tongue and a scaly dermatitis of the scrotum in Southern India. Oral or parenteral administration of 5 mg. to 15 mg. of riboflavin daily cleared up the condition in a week or so.

Tisdall, McCreary and Pearce [237] comment on the high incidence of corneal vascularization in the personnel of the Royal Canadian Air Force, whom they estimate to have a daily riboflavin intake of 1.6 mg. The eyes of a hundred and sixty-eight men were classified as follows :—

Normal eye	0.5 per cent.
Stage 1	8.6 „ „
Stage 2	43.9 „ „
Stage 3	46.9 „ „

A normal eye was considered to be one in which no proliferation of the vessels of the limbic plexus and no penetration of the cornea occurred. In Stage 1 there was proliferation of the vessels of the limbic plexus only. In Stage 2 vessels of the limbic plexus showed proliferation and penetrated the cornea with twigs and streamers. In Stage 3 the eye showed Stages 1 and 2 and the cornea was penetrated with twigs, streamers and capillary loops. As Stannus [240] points out, it is quite impossible, owing to the great variability, to formulate a scheme for describing the degree of vascularity. Many of the men suffered from tiredness and aching of the eyes; watering of the eyes; gritty feeling under the lids; headaches; intolerance to reading and decreased visual acuity. Riboflavin in doses of 9.9 mg. daily for two months caused a progressive decrease in the corneal vascularity of seventy per cent. and symptomatic improvement in ninety per cent. Progress was recorded by means of photographs taken with a special camera.

In a later study McCreary, Nicholls and Tisdall [279] observed corneal vascularization in Air Force personnel receiving 2.9 mg. of riboflavin daily for a year. Supplements bringing the intake up to 6.2 mg. daily failed to produce any consistent change in corneal vascularization.

It has been assumed by Bessey and Wolbach [41] that corneal vascularization occurs in riboflavin deficiency as a compensatory mechanism to bring the blood into closer contact with the corneal epithelium. This being normally avascular contains no hæmin compounds, and probably the oxidation of metabolites within it occurs through the agency of the riboflavin enzyme system, the riboflavin diffusing in from the limbic plexus according to Bessey and Wolbach. They assume that in ariboflavinosis blood vessels grow into the cornea in an attempt to overcome the local anoxia by bringing available riboflavin into closer proximity with the corneal cells. This view has been widely accepted. The work of Philpot and Pirie [250] makes this explanation unlikely. They have shown that whereas the cornea contains less riboflavin than any other ocular tissue (0.2 µg per gram), the lacrymal gland contains as much as 6.5 µg per gram. They therefore suggest that the cornea receives its riboflavin from the lacrymal secretions rather than from the blood of the limbic plexus.

Validity of Circumcorneal Injection and Corneal Vascularization as Manifestations of Riboflavin Deficiency. After the observations of Sydenstricker and his colleagues in 1940 on the eye symptoms of ariboflavinosis, circumcorneal injection and corneal vascularization were accepted as certain diagnostic signs of the condition and were used in nutrition surveys as an index of riboflavin nutrition [289]. Since 1942, however, many investigations have shown that neither of these signs is diagnostic of riboflavin deficiency, although they may occur in riboflavin deficient subjects. According to the American workers there is an avascular zone between the limbic plexus and the corneo-scleral junction (Fig. 64). In the early stages of riboflavin deficiency "circumcorneal injection" is stated to occur, *i.e.*, the limbic plexus becomes engorged and congested. In the next stage it is stated that small vascular twigs or streamers run out from the limbic loops through the avascular region towards the cornea, and that these twigs in turn anastomose with one another to form loops from which further streamers are formed so that the cornea is extensively vascularized (Figs. 65, 66).

Is "circumcorneal injection" pathognomonic of riboflavin deficiency? Stannus [240], who has examined between four thousand and five thousand eyes by slit-lamp microscopy for signs of ariboflavinosis (or, as he prefers to call it, hypo-riboflavinosis), states that there is not a narrow avascular zone between the limbic plexus and the corneo-scleral junction as maintained by Kruse, and that he has seen both circumcorneal injection and corneal vascularization without any evidence of riboflavin deficiency. He states that the area between the limbic plexus and the corneo-scleral junction may appear avascular because the blood vessels may be constricted and empty. They become visible, however, on using drugs such as dionine, which dilate the vessels. The limbic plexus becomes congested and engorged on the slightest provocation. Engorgement occurs in all varieties of conjunctivitis, in those whose eyes are exposed to heat and dust, cold wind, bright light, mild infection, chemical irritants and even by rubbing the eye [241, 242]. Hence circumcorneal injection is of no value in the diagnosis of riboflavin deficiency.

Controlled experiments were made by Scott [257], who divided a hundred and twenty patients with corneal vascularization into two groups, one being treated with riboflavin and the other serving as a control. The percentages in which improvement, no change and deterioration were observed were the same in the treated as in the non-treated group. Similar observations were made by McCreary, Nicholls and Tisdall [279], who failed to observe any improvement in corneal vascularization in subjects receiving 6.2 mg. of riboflavin daily. Scott also observed that in a group of over five hundred Europeans in Gambia, thirty-seven per cent. had some degree of corneal vascularization, while in a group of natives only five per cent. were affected, although the incidence of cheilosis, angular stomatitis, glossitis and other signs of riboflavin deficiency was twice as great in the natives as in the Europeans.

Scarborough [205] examined two-hundred-and-four unselected out-patients between the ages of twelve and sixty-nine in Edinburgh and noted circumcorneal injection in forty-three, that is, in thirty-four per cent. In

patients aged fifty or over the incidence was sixty-eight per cent. The administration of riboflavin had no effect on the condition except in five cases out of eight suffering from frank vitamin deficiency.

Vail and Ascher [249], from observations on over seven hundred patients in the Nutrition Clinic, Birmingham, U.S.A., conclude that no relationship whatsoever exists between any type of vitamin deficiency and vascular congestion in the limbal region. Actually more cases were seen in better fed than in poorly fed patients.

Corneal vascularization has been found to be fairly common in the population at large. Sandstead [248] found the condition extremely common in school children and young adults in America, the incidence ranging from eighty per cent. to ninety-three per cent. in the groups studied. Doses of 5 mg. of riboflavin daily caused no significant change in the degree of vascularization.

Ferguson [242] found that of two hundred and fifty patients at the Royal Infirmary, Sheffield, seventy-six had abnormal corneal vascularization, but only seventeen were possibly due to riboflavin deficiency. Ten of these were stated to respond to 10 mg. of riboflavin in three to four weeks. From a study of twelve hundred subjects, Youmans and his collaborators [254] concluded that corneal vascularization bears no relationship to the dietary intake of riboflavin, nor to other evidence of riboflavin deficiency.

An extensive survey of the degree of corneal vascularity was made by Lyle, Macrae and Gardiner [244] in four thousand R.A.F. personnel in Great Britain. Even among some well-fed subjects blood vessels were seen in the cornea and many subjects with corneal vascularization failed to improve when the diet was supplemented or when they were given riboflavin. It was concluded that corneal vascularization may result from a nutritional defect, but that riboflavin deficiency is not the cause in most cases. Indeed the results suggested that other unknown factors in fruits and vegetables affect the condition more than riboflavin. Liver, nicotinic acid, pantothenic acid, vitamin B₆, vitamin A and ascorbic acid, did not prevent or cure corneal vascularization.

Some other conditions in which corneal vascularization may occur are vitamin A deficiency [41], tryptophane deficiency [280], injury to the corneal epithelium by chemical irritants, diseases causing pannus, such as trachoma, phlyctenular keratitis, and any superficial keratitis [241]. The instillation of a simple irritant, such as five per cent. soap solution, into the conjunctival sac may cause collapsed afunctional blood vessels in the cornea to become engorged [279]. Another observation that requires some explanation is that of Boehrer, Stanford and Ryan [199], who failed to observe corneal vascularization in volunteers on diets low in riboflavin (approx. 0.5 mg. daily), yet it appeared in a control subject receiving 3.5 mg. of riboflavin daily.

It may therefore be concluded that both circumcorneal injection and corneal vascularization observed for the first time in an examination are not specific signs of riboflavin deficiency. When they do occur with other manifestations of riboflavin deficiency—angular stomatitis, glossitis, seborrhœic dermatitis, genito-anal lesions, and other ocular symptoms—

they may be part of the ariboflavinosis syndrome as they regress on treatment with riboflavin.

Neurological Manifestations of Riboflavin Deficiency. American writers have not included neurological manifestations in the ariboflavinosis syndrome. Workers in the English colonies who wrote before the recognition of riboflavin as a factor in human nutrition, described certain neurological symptoms which did not fit into the clinical picture of beriberi or pellagra. Stannus [240] is inclined to believe that they are the result of riboflavin deficiency, with the possibility that lack of another factor of the B complex may be involved. Scott [189], during an investigation of natives in Jamaica, included a central neuritis and a burning sensation of the feet in his symptomatology. This burning sensation of the feet is also mentioned by other writers, such as Landor and Pallister [248], who also observed pains and weakness of the legs, rombergism, exaggerated knee jerks, "stocking" anæsthesia, and retrobulbar neuritis followed by partial optic atrophy. The appreciation of touch, temperature and deep sensation were also affected and in late cases the authors remarked that the symptoms resembled those of subacute combined degeneration of the cord. Grande Covián and Jiménez García [251], writing in 1940 after the Spanish Civil War, noted the association of glossitis and cheilosis with loss of visual and auditory acuity, paræsthesiæ, a burning pain in the soles of the feet and ataxia. Moore [246, 247] observed that in his early cases complaining of sore lips and tongue there were no changes in the fundi, but later pallor of the temporal halves of the discs was noted, in some optic atrophy, in others signs of optic neuritis. Central scotoma and some retraction of the visual fields were also present. This was also noted by Wilkinson and King [256, 301]. The nervous symptoms that Moore described were mental dullness, muscular weakness, ataxia and paræsthesiæ.

Stannus [240] accepts these symptoms as manifestations of riboflavin deficiency and to them he has added others which he has observed. These fit into a condition resembling the "cerebellar syndrome"—muscular asthenia and hypotonia, ataxia, jerky clonic contractions, dysmetria, dysidiadokokinesis, nystagmus, vertigo, tremor, typical gait and pendulum knee jerks. The patient shows mental apathy, hyperemotionalism and a Parkinson-like facies. Cerebellar manifestations, including ataxia, dysmetria, and decomposition of movement, have been noted by Ransome [808] in Singapore coolies suffering from ariboflavinosis.

Pathogenesis of Ariboflavinosis. Stannus [240] believes that the varied lesions of riboflavin deficiency are a manifestation of an acute functional derangement of the capillary circulation of the affected parts. The first tissue to suffer from riboflavin deficiency is the endothelium of the capillary system. The capillaries undergo a reversible functional disturbance—a capillary dysergia—resulting in loss of tone and dilatation. Normal cellular metabolism is upset and tissue functions are disturbed. The first tissues to suffer are those with the largest number of capillaries, those whose metabolism is greatest, and those with a very specialized function. The interference with tissue cell metabolism is probably of the nature of an anoxia, using this term in its widest sense. If the anoxia is not too prolonged recovery of function takes place in the capillaries when supplied

with adequate riboflavin, otherwise irreversible processes leading to pathological changes occur. According to Stannus the skin lesions of ariboflavinosis, which occur where the skin is thin or highly specialized and at the mucocutaneous junctions about body orifices (lips, palpebral fissures, nares, prepuce, vulva, anus, scrotum) are due to capillary congestion, with resulting impaired nutrition of the skin. The colour of the magenta tongue (p. 335) too is the result of capillary dilatation and a sluggish blood flow. While Stannus grants that the changes in the cornea are due to anoxia following lack of riboflavin, he believes that the visual disturbances have no immediate connection with the eye, but are part of a lesion in the central nervous system. The neurological manifestations are the expression of a metabolic disturbance in nerve tissue produced by capillary dysergia, the more vascular tissues being the first to suffer, *e.g.*, the grey matter and the cerebellar neuropyl.

Diagnosis of Ariboflavinosis. The diagnosis of ariboflavinosis is made on the history, clinical examination and response to treatment with riboflavin. Laboratory tests have proved disappointing. Blood and urine studies have been made and saturation tests have been devised for the laboratory diagnosis of riboflavin deficiency, but they are of doubtful value and have not received general acceptance as normal standards have not been laid down (p. 323). It is generally accepted that ariboflavinosis cannot be determined by single estimations of the twenty-four-hour excretion of riboflavin [126]. It is also possible to depress the excretion of riboflavin to zero without producing evidence of deficiency symptoms [126]. Feder, Lewis and Alden [302] state, however, that an excretion of 0.53 to 0.8 μ g of riboflavin per c.c. of urine denotes an adequate intake, while values below 0.8 μ g per c.c. denote a deficient intake of riboflavin. Gastric achlorhydria is a common finding, and was noted by Sydenstricker in fifty per cent. of his patients [252], although it is, however, non-specific.

In the clinical examination the characteristic seborrhœic facial lesions, the glossitis, and the lesions in the region of the anus, vulva and prepuce are of diagnostic importance, but the ocular lesions, such as circumcorneal injection and corneal vascularization, although they may be present are non-specific (p. 347). Angular stomatitis may be present, but it may be due to causes other than ariboflavinosis (p. 331). The neurological lesions of diagnostic value are burning of the feet, retrobulbar neuritis followed by partial optic atrophy, loss of visual acuity, paræsthesia and anæsthesia in the feet and legs, and the cerebellar syndrome of Stannus (p. 349). A poor dietary history—one lacking meat, cheese, eggs, green vegetables, milk—is suggestive. Diagnosis may be assisted by the therapeutic test. If the lesions do not show some signs of resolution in a few days after giving 10 mg. of riboflavin daily, the condition is not due to riboflavin deficiency.

A fatal case of acute riboflavin-deficiency in man has been reported [114], and Sydenstricker [86] has also entertained the possibility of this. Acute riboflavin deficiency has been produced experimentally in dogs, characterized by muscular weakness, ataxia, changes in the electrocardiogram, collapse and death, with a fatty liver post-mortem [49, 50]. Field and Wise [114] have recorded similar findings in patients with chronic diseases

who have died suddenly. Although there is no clear cut evidence of acute ariboflavinosis, the possibility of its existence might be borne in mind.

In the differential diagnosis of ariboflavinosis, pellagra, sprue, idiopathic hypochromic anaemia, subacute combined degeneration, disseminated sclerosis, and cerebellar lesions must be considered. Glossitis, lesions of the lips, anus, scrotum and vulva, burning feet, muscular weakness and retrobulbar neuritis may occur in pellagra. Angular stomatitis and skin lesions similar to those seen in ariboflavinosis have been reported in sprue and idiopathic hypochromic anaemia [108]. As Landor and Pallister [248] pointed out, the neurological symptoms in the late stages of what is now known as ariboflavinosis resemble those of subacute combined degeneration (paræsthesiæ of legs, ataxia, impaired appreciation of touch, pain and temperature, rombergism, exaggerated knee and ankle jerks, nystagmus, achlorhydria, glossitis). Disseminated sclerosis may also be confused with some of the neurological symptoms reported in ariboflavinosis (retrobulbar neuritis, misty vision, paræsthesiæ, muscle weakness, nystagmus, vertigo, emotional changes, exaggerated reflexes). Lastly the neurological manifestations listed by Stannus (p. 349) are almost identical with those of the cerebellar syndrome.

Treatment of Ariboflavinosis. Most cases respond to treatment with riboflavin in doses of 5 to 15 mg. by mouth daily. The average case responds to 5 mg. by mouth daily [115], although Jolliffe [89] prefers to give 50 mg. intramuscularly at the beginning of treatment for a few days, followed by 10 mg. daily by mouth. If the patient suffers from achlorhydria, vomiting, diarrhoea, hepatic disease or other disorder preventing absorption or utilization, the riboflavin is given parenterally in doses of 10 to 15 mg. of the sodium compound [92, 115]. Jolliffe [258] administers 10 to 20 mg. daily by mouth in mild to moderate cases of ariboflavinosis and 20 to 50 mg. in severe cases. Larger doses, although wasteful, are non-toxic [81]. Yeast in doses of 60 to 90 gm. daily is also curative [252]. Sydenstricker [86] warns against giving large doses of a single vitamin for long periods in the treatment of avitaminosis, as although it may cure the major manifestations of the condition under treatment, it may precipitate a deficiency of another member of the vitamin B complex (p. 248). He therefore gives other members of the B complex such as yeast, crude liver extract, wheat or rice bran extract with the riboflavin. Spies and his co-workers [255] consider that 3 to 5 mg. orally a day is sufficient in the treatment of the average case of ariboflavinosis.

Some workers believe that the daily dose should be divided into three to five doses to maintain a constant level in the tissues and prevent rapid excretion. In addition to vitamin therapy the diet must include generous amounts of food rich in the vitamin B complex, *e.g.*, whole grain cereals, meat, milk, liver, eggs and cheese, otherwise the patient will relapse as soon as riboflavin is withdrawn.

Following the administration of riboflavin, photophobia, burning, itching and blepharospasm are relieved in twenty-four to forty-eight hours and visual acuity slowly improves. Oral lesions begin to improve in three days or so, but complete resolution may take weeks [91, 92]. Where

circumcorneal injection is present the vessels become blanched in one to two days, and in cases of corneal vascularization the capillary loops empty and become occluded within five to eight days of commencing treatment. The superficial opacities and nebulae described by Sydenstricker also clear up, but more slowly than other ocular lesions.

The improvement in the glossitis can be followed by tongue prints. The tongue is wiped dry and covered with ink by means of an inking pad. Then stiff white glossy paper is placed on the tongue with a rolling motion and quickly removed. Serial tongue prints so obtained are valuable in following the type and progress of the glossitis (Figs. 100 to 105).

There is no information on the speed with which the neurological manifestations attributed to ariboflavinosis resolve.

Sprue, Pellagra and Pernicious Anæmia. These are three separate disease entities, but there is good reason for believing that they are allied nutritional diseases. There are many symptoms common to all of them, *e.g.*, gastro-intestinal symptoms, neurological manifestations and a macrocytic anæmia. A patient with achlorhydria, stomatitis, glossitis, diarrhoea, mental depression, involvement of the lateral and posterior columns of the spinal cord and severe anæmia, but no cutaneous lesions, may be suffering from any one of them. If in addition there is a characteristic symmetrical bilateral exfoliative dermatitis of the hands and feet a diagnosis of pellagra may be made; if the anæmia responds to injections of liver and returns on withdrawing the latter, pernicious anæmia is diagnosed; while the passage of large, fatty, fermenting and pasty stools confirms the diagnosis of sprue. The country in which the patient lives is sometimes of help in differential diagnosis. Thus if the first-mentioned group of symptoms occurs in an area in which pellagra is endemic, this may be diagnosed even in the absence of dermatitis. Pernicious anæmia is rare in the tropics [260]. Gastro-intestinal symptoms may occur in pernicious anæmia—sore tongue, sore mouth, epigastric distress, diarrhoea, anorexia, nausea, achlorhydria—so that sometimes a differential diagnosis between pernicious anæmia and sprue may be difficult without laboratory tests such as the quantitative estimation of fats in the stools. A histamine resistant achlorhydria is always present in pernicious anæmia, but it occurs only in about thirty per cent. of cases of sprue. The sprue patient, however, eliminates neutral red administered parenterally by the gastric mucosa, but the pernicious anæmia patient does not [258]. It is a significant fact that in sprue, pellagra and pernicious anæmia the one effective treatment is liver and a high protein, low carbohydrate, rich vitamin diet. Sir Philip Manson-Bahr [129] has also found nicotinic acid effective in the treatment of the glossitis of sprue. Fatty degeneration of the liver may occur in all three conditions. Harris and Harris [118, 259] believe that liver insufficiency is a factor in all of them, and they state that they have seen the three conditions occurring in the same patient.

Sir Philip Manson-Bahr [129, 260] has put forward the suggestion that sprue, pernicious anæmia and pellagra are deficiency diseases due to lesions in the gastro-intestinal tract interfering with the absorption or causing the destruction of certain essential vitamins. Gastro-duodenal inefficiency, resulting in the absence of the intrinsic factor of Castle produces the

pernicious anæmia syndrome; jejuno-ileal inefficiency produces the sprue syndrome; and ileocolic insufficiency the pellagra syndrome. By inefficiency Sir Philip Manson-Bahr means the inability of the absorptive mucosal surface to perform its intricate and proper functions. The glossitis common to the group may be due to a deficiency of riboflavin and nicotinic acid.

Since the postulation of the intrinsic and extrinsic anti-pernicious anæmia factors by Castle, it has been thought that the extrinsic factor might be one of the B vitamins. Riboflavin has been suspected, but Castle [285] has definitely shown that neither riboflavin nor any of the chemically identified members of the vitamin B complex is effective in producing reticulocytosis in pernicious anæmia patients. Nevertheless, Castle still regards the extrinsic factor as an unidentified thermostable component of the vitamin B complex.

Sprue. Sprue is pre-eminently a disease common to the Europeans living in warm climates, although it is rare in tropical Africa [260]. Sprue has been reported in England, Scandinavia, United States and Canada, and in individuals who have never been to the tropics (non-tropical sprue, or idiopathic steatorrhœa). Sprue has also been described in native races such as Chinese, Javanese, Malays and Cinghalese.

Ætiology of Sprue. The ætiology of sprue is still a matter of discussion. It is now generally agreed that tropical sprue, non-tropical sprue or idiopathic steatorrhœa, and celiac disease are varieties of the same disorder—the sprue syndrome [73]. Originally supposed to be due to a monilia infection, sprue is now believed to be a deficiency disease. "Surgical sprue" associated with defective food absorption following gastro-enterostomy has been described [294]. The fact that sprue responds to treatment with liver, which is rich in the vitamin B complex, and that some of the symptoms are alleviated by riboflavin and nicotinic acid, suggests that a deficiency of one or more members of the vitamin B complex may be involved in its ætiology [79, 129]. Liver extract and crude preparations of the B complex are also stated to be effective in the treatment of celiac disease [299, 300].

Stannus [261] has advanced the view that the deficient absorption of fat and carbohydrate that occurs in sprue is primarily due to failure of phosphorylation, which is essential for the absorption of fat and carbohydrate by the intestinal mucosa. The "physiological lesion" of sprue is thus failure of phosphorylation according to Stannus. The enzymes which catalyse phosphorylation—the phosphate carriers—probably have as coenzymes some member or members of the vitamin B complex (riboflavin, nicotinic acid, vitamin B₆). This hypothesis of Stannus receives some support from the observations of May and his co-workers [262] who have shown that the absorption of fat and glucose, the intestinal motility, and indeed the whole clinical course of celiac disease are favourably influenced not only by crude liver extracts given parenterally but also by mixtures containing vitamin B₁, riboflavin, nicotinic acid, pantothenic acid and pyridoxine given parenterally. Oral therapy was unsatisfactory. It is known that both riboflavin and codehydrogenase I, which contains nicotinic acid (p. 381), may function in the transfer of phosphoric acid.

Pathology of Sprue. The principal post-mortem findings in patients dying of sprue are extreme emaciation, pallor and marked atrophy of many organs, an erythromegaloblastic hyperplasia of the bone marrow (cf. pernicious anæmia) and disappearance of the valvulæ conniventes of the small intestine. Many of the post mortem findings, however, have little bearing on derangements of function underlying the symptoms seen during life. In chronic sprue the muscles are wasted, visceral fat largely absent, and the heart small and atrophied. Considerable attention has been paid to the appearance of the intestine, but the appearance at autopsy may well be due to post mortem changes or secondary changes occurring in a wasting disease. Thinning and atrophy of the small intestine and progressive degeneration of the absorptive and secretory tissues have been described [260]. These changes, if they are not post mortem ones are reversible, as the radiological appearances of the intestine suggest that recovery occurs under treatment.

Symptomatology. The symptoms, of which there are an infinite variety, may be broadly classified under oral, gastro-intestinal, hæmatological, cutaneous and mental. The disease lasts for a number of years with alternate exacerbations and remissions. It begins insidiously with periods of diarrhœa, stomatitis and glossitis, and in a well established case the stools are characteristic, the abdomen is distended and there is progressive emaciation and a characteristic anæmia.



FIG. 68. Sprue. Characteristic appearance of the tongue in a chronic case. It is "clean," raw, very smooth, and red.

Oral Lesions. The tongue is raw, red and burning with superficial erosions, patches of congestion, and vesicles on the sides and tip. Ulcers may form at the level of the rear molars.

Later the tongue becomes smooth, due to atrophy of the papillæ, fissured and indented by the teeth (Fig. 68). Acid, hot and spiced foods cause intense pain, and as a result of the irritation caused by the lesions salivation is profuse and dribbling occurs owing to the pain produced by swallowing. Ulceration of the buccal and gingival mucous membrane with pain and burning of the whole oral cavity and angular stomatitis occur in severe cases. The vesicles on the tongue are said to be characteristic, although they have to be distinguished from aphthous ulceration occurring in other conditions. Tongue and mouth lesions are present in seventy per cent. of the cases [260].

Gastro-intestinal Symptoms. Dyspepsia and diarrhœa are prominent and constant features of sprue. There is sometimes dysphagia, suggesting

that the mucous membrane lesions of the mouth are continued down to the œsophagus. Dyspepsia is manifested by a feeling of weight, oppression and distention after food. Meteorism, flatulence and nausea are common and abdominal cramps are sometimes complained of. Some patients complain of a burning feeling along the entire gastro-intestinal tract from mouth to anus.

Diarrhœa in the acute form is frequently watery ("dirty dish water"), pale and fermenting with undigested food and fatty acids. The frequent acid motions may cause excoriation of the anal margin and perianal skin. In the chronic case the stools are classically bulky, pasty, pale-grey,

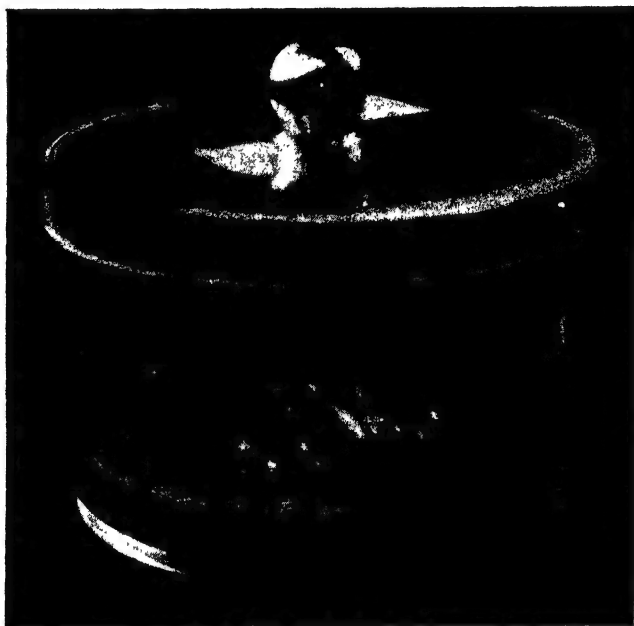


FIG. 69. Typical sprue stool. This is copious, pale, pasty and frothy, due to excessive fermentation.

frothing from the presence of intestinal gas, and particularly foul-smelling (Fig. 69). There is much fat and fatty acid crystals on microscopical examination. Actually the appearance of the stools is variable and the best evidence of steatorrhœa is the estimation of fat in the stools. Shrinkage of the liver, as shown by diminution of liver dullness is present in over half the cases [260].

Hæmatological Manifestations. After the diarrhœa a characteristic anæmia presents itself, indistinguishable hæmatologically from pernicious anæmia. Although anæmia is a common finding, it is not pathognomonic. It is macrocytic and hyperchromic. Erythrocyte counts of 1.5 to 1 million are not uncommon with a colour index of 1.5 or more. The blood picture explains the weakness and breathlessness seen in some patients. In non-tropical sprue the anæmia may be macrocytic and hyperchromic, but is

often normocytic or hypochromic microcytic, suggesting that iron deficiency plays some part. The leucocyte count is usually normal or reduced and pronounced leucopenia is not uncommon. A blood crisis in



FIG. 70. Hæmorrhagic Symptoms in a Patient with Sprue. Petechiæ can be seen around the hair follicles.

sprue may result in a fatal issue. The most thorough and detailed study of the hæmatology of sprue is that of Rodriguez-Molina [263], published in 1939 and based on a hundred cases. Stained blood films showed in



FIG. 71. Sprue. Section of skin stained by silver technique, showing heavy deposits of pigment in the rete and basal cell layers.

nearly all cases polychromasiâ, basophil stippling, nuclear remnants and nucleated forms—the changes seen in pernicious anæmia. Bone marrow studies show the same megaloblastic hyperplasia as in the latter disease. The response of the anæmia of sprue to the administration of liver is likewise comparable to that of pernicious anæmia. An increase in reticulocytes is followed by an increase in red cells and hæmoglobin.

A hæmorrhagic diathesis with large subcutaneous ecchymoses (Fig. 70), hæmatomata, hæmarthroses and abnormal bleeding from the various orifices of the body has been noted in a few cases of severe sprue. This was formerly regarded as a scorbutic manifestation, but following the discovery of vitamin K and its relation to prothrombin the nature of the severe hæmorrhages that may occur in sprue have been clarified. The hæmorrhagic tendency is due to a low blood prothrombin, resulting from a diminished intake, absorption or utilization of vitamin K (p. 811).

Cutaneous Lesions. In a high percentage of cases of sprue the skin is rough, loose and dry as well as pigmented [264]. The pigmentation is brownish, diffuse or patchy, with a preference for the forehead, nose, malar region, abdomen and legs. Kaufman and Smith [264] have studied the histopathology of this pigmentation and report an increase in the melanin content of the basal cell and rete lateres (Fig. 71). This may occur in Addison's disease.

Other Manifestations of Sprue. Many other symptoms may be seen in

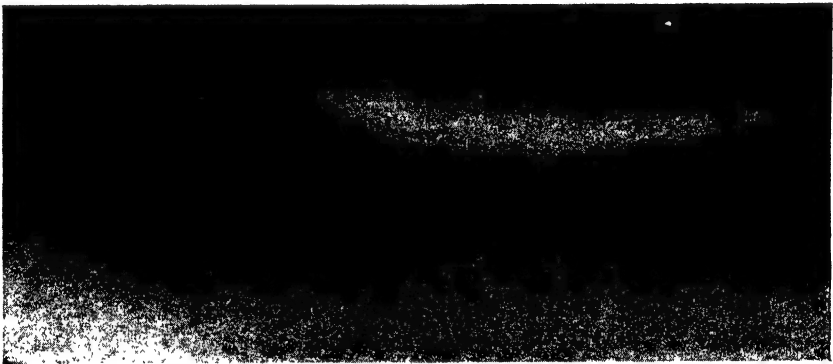


FIG. 72. Tetany in a Patient with Sprue.

a case of sprue. Loss of weight, with muscle wasting and loss of fat depots, is a constant finding. It may be so severe as to lead to emaciation. Muscular cramps and tetany are frequent in well-marked cases. Cramps occur in the legs, particularly in sleep, and tetany in the hands and feet (Fig. 72). Tetany is the result of the low blood calcium. A low-grade fever is not uncommon in severe cases of sprue, and the blood pressure is often low (*e.g.*, 90 to 100 mm. systolic) and the basal metabolic rate reduced by ten to twenty per cent. The mental disturbances in sprue are said to be characteristic and of diagnostic aid to the nursing staff. The patient is irritable and cross, and his mental condition seems to run parallel with that of his bowels.

Clinical Pathology. Achlorhydria and hypochlorhydria are frequently met with, but achlorhydria after an injection of histamine is uncommon. In this respect the gastric secretion differs from that in pernicious anæmia, in which true achylia gastrica occurs.

Bile acids and pancreatic enzymes are present in the duodenal juice so that the malabsorption of fat cannot be due to lack of bile salts and

pancreatic enzymes as formerly thought. In sprue the fats are split, but the split fat is not absorbed and is excreted in the faeces, sixty per cent. or more of the dry weight of which may be fatty acid. There is also considerable unabsorbed carbohydrate in the intestine. The fermentation of this accounts for the flatulence, meteorism and frothy stools.

A flat blood sugar curve following the ingestion of glucose, indicating a low glucose tolerance, is a characteristic finding in tropical and non-tropical sprue and in coeliac disease. A rise of less than 40 mg. per 100 c.c.



FIG. 78. X-ray of Normal Intestine three hours after Barium Meal. Note the regularity of deposits of barium in the mucous folds of the jejunum and the greater filling of the coils of ileum compared with Figs. 74 to 77.

above fasting level after ingesting 1 gm. of glucose per kilo of body weight is common. The flat curve is due to delayed or defective absorption of glucose as an intravenous glucose tolerance test gives a normal curve.

Blood calcium and phosphorus are lower than normal. This is because (a) calcium is lost in the intestine by the formation of insoluble soaps with the free fatty acids, (b) vitamin D deficiency results from imperfect fat absorption.

Striking X-ray changes (Figs. 73 to 77) are seen in sprue after a barium meal [102, 265-268]. The principal changes are : segmental distribution of the barium ; irregular dilatation of the small and large intestine ; dis-

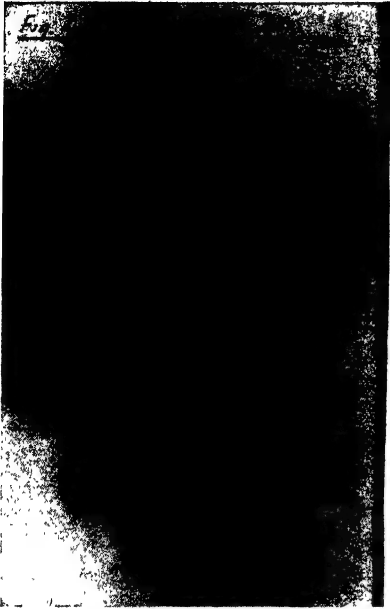


FIG. 74. Idiopathic Steatorrhœa. X-ray fifteen minutes after barium meal. The flow of barium in jejunal coils is regular at this stage.

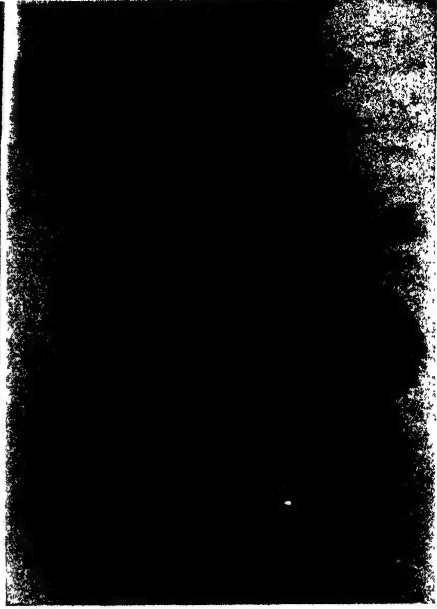


FIG. 75. Idiopathic Steatorrhœa. X-ray one hour after barium meal. The coils of jejunum are irregularly dilated and the barium broken up into irregular segments.



FIG. 76. Idiopathic Steatorrhœa. X-ray one and a half hours after barium meal. The coils of jejunum and ileum are still irregularly filled.

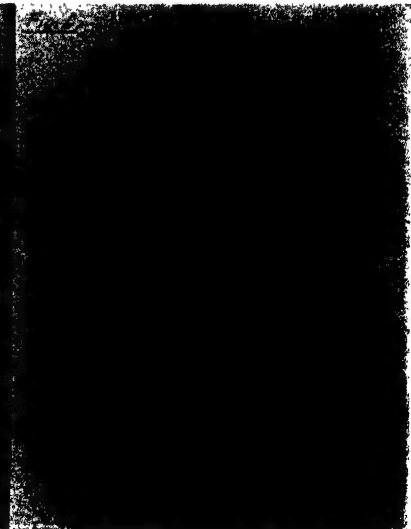


FIG. 77. Idiopathic Steatorrhœa. X-ray two hours after barium meal. The disposition of the barium is still irregular and there is delayed emptying.

tortion of the mucosal pattern of the gut, particularly in the jejunum, where the valvulae conniventes have an "iron-out" appearance; and delayed emptying. Another important X-ray finding is occlusive spasm of the gut. The bones do not exhibit a compact cortex and show signs of decalcification (Fig. 78). Marked improvement in the radiological picture occurs after treatment with liver or the vitamin B complex. Ingelfinger and Moss [269] have shown that the gut in sprue lacks the usual resistance to distension exhibited by the normal intestine, and observations after injecting parasympathetic stimulants suggest that in sprue the nervous apparatus of the small intestine fails to liberate sufficient acetylcholine.

Gastroscopy and sigmoidoscopy show in many cases a beefy-red,

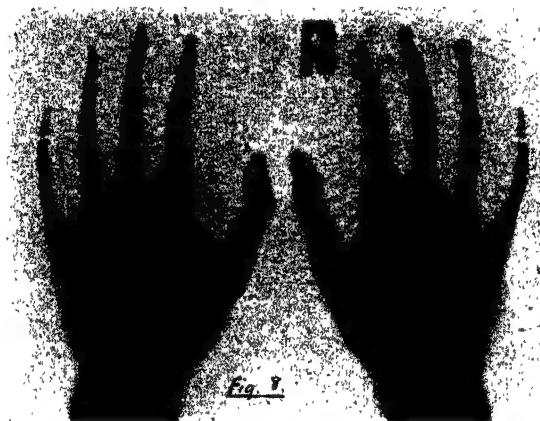


FIG. 78. Idiopathic Steatorrhœa. X-ray of hands, showing absence of compact cortex in the bones and signs of decalcification.

glossed, hyperæmic mucosa in keeping with the appearances of the tongue and mouth.

Differential Diagnosis. The most likely conditions to be confused with sprue are pellagra, pernicious anæmia, chronic infectious dysenteries, pancreatic steatorrhœa, Addison's disease and diseases of the mesenteric glands. The diagnosis of sprue is made on the presence of steatorrhœa, loss of weight, low glucose tolerance curve, anæmia, achlorhydria, glossitis and radiological findings.

The differential diagnosis between pernicious anæmia and sprue is given on p. 352. In addition high van den Bergh serum bilirubin readings are exceptional in sprue, but the rule in pernicious anæmia. Clinically it may be difficult to distinguish sprue from pellagra; laboratory tests help. Chronic infectious dysentery is differentiated by recognition of the causal organism in the stools. Pancreatic steatorrhœa is distinguished by the presence of large quantities of neutral unsplit fat in the fæces; there is no pancreatic failure in sprue. Pigmentation, asthenia and hypotension in a patient with sprue may be mistaken for Addison's disease; estimation of the plasma electrolytes help to differentiate the latter. Diseases of the

mesenteric glands (mesenteric lymphatic obstruction, tuberculosis, lymphadenoma) may resemble sprue because of the wasting, diarrhoea with light-coloured fatty faeces and distended doughy abdomen. Laboratory tests of faeces, blood and gastric juice and the glucose tolerance test help in the diagnosis.

Treatment. Rest, good nursing and diet form the sheet anchor of treatment. Complete rest in bed is essential in severe cases. The diet should be high in protein and monosaccharides (glucose, fructose), but low in fat, starches and other polysaccharides, which cannot be assimilated. Milk, eggs, raw red meat, fruit and liver are the most valuable articles in the dietary, although if these cannot be tolerated, pounded meat, bananas and skimmed milk may be given. The first phase of feeding may have to be restricted to fruit juices, gelatin and egg albumin. Crude sources of the vitamin B complex such as yeast in any form, yeast extract, rice polishings, and dried liver or liver extract are valuable. Liver extract given parenterally and not orally has been found to be necessary for complete relief of symptoms. Five to 15 units are given daily until symptomatic improvement occurs, and then the dose is reduced. The maintenance dose of liver extract is worked out for each patient, the criterion being the cure of intestinal dysfunction rather than restoration of a normal blood picture. Even after apparent cure liver injections should be given periodically. Bananas and strawberries, raspberries and other fruits are useful because they supply not only vitamins, but glucose in an assimilable form. As many as twelve to sixteen bananas and two pounds of strawberries can be taken daily. A high protein milk powder (Sprulac) has been devised by Fairley. This combines the advantages of a high protein food with the bland properties of milk. As improvement sets in fish and cheese may be added to the diet, then bread and vegetables with a low carbohydrate content and finally cereals, butter and other fats in small quantities only.

Sir Philip Manson-Bahr [79], who has treated a number of cases of tropical sprue with 150 to 300 mg. of nicotinic acid and 3 mg. of riboflavin daily, finds that these vitamins have a striking effect in causing the disappearance of the stomatitis and glossitis. In his opinion they also cause improvement in the intestinal symptoms; the diarrhoea ceases, the stools become normal in size and colour, and the meteorism disappears. The beneficial effect of riboflavin was particularly noticeable in cases with angular stomatitis and an atrophic condition of the perianal skin. Sir Philip Manson-Bahr [129] claims that nicotinic acid in doses of 300 mg. daily, combined with liver therapy constitutes the best method of curing sprue, thereby indicating a link between it and pellagra. He regards the sprue syndrome as a deficiency disease mainly due to the non-absorption or destruction of nicotinic acid and riboflavin in the small intestine.

Vanotti [64] and Pock-Steen [78] claim that riboflavin alone improves some cases of sprue.

Patients with hypochromic microcytic anæmia should also receive ferrous sulphate 0.2 to 0.4 gm. three times daily. In cases of very severe anæmia blood transfusion may be necessary. Calcium salts and vitamin D may be necessary in patients with tetany, muscular cramps and osteo-

porosis and also vitamins A and K if there is any clinical evidence of a deficiency of these. The addition of lecithin to the diet improves the absorption of vitamin A [269].

Plummer-Vinson's Syndrome. This syndrome is characterised by glossitis, anæmia, dysphagia and achlorhydria and is practically confined to women. It has been ascribed to ariboflavinosis [142, 225] on account of some resemblance in the symptomatology of the two conditions, although according to one report riboflavin is ineffective in the treatment of the Plummer-Vinson syndrome [306]. Pellagra has many symptoms in common with Plummer-Vinson's syndrome as well. Sjögren's syndrome (filiform keratitis and dryness of the mucous membranes) is believed by Franceschetti [101] to be due to riboflavin deficiency. The increased sedimentation rate and the frequent involvement of joints suggests, however, an infective ætiology.

Clinical Uses of Riboflavin

Apart from its use in the treatment of definitely diagnosed deficiency diseases the value of riboflavin in therapeutics has yet to be shown. There is some evidence that some other vitamins, such as nicotinic acid and vitamin B₁, exert a pharmacological action in conditions in which no vitamin deficiency exists, but it has not yet been shown that riboflavin has a pharmacological as distinct from a vitamin action.

Johnson and Eckhardt [98] noted the resemblance between acne rosacea and the flushing of the face and prominence of capillary dilatation on the cheeks and nose of some of their patients with ariboflavinosis. They therefore suggested that acne rosacea is due to riboflavin deficiency, the persistent congestion and redness, telangiectases, seborrhœic hyperactivity with dilated follicles, and thickening and hyperactivity of the subcutaneous tissue being part of the physiological response of the blood vessels to deficient oxidative reactions (*cf.* Stannus' theory of capillary dysergia, p. 349). Johnson and Eckhardt claim that the administration of riboflavin responds to treatment with 1 to 2 mg. of riboflavin daily, although Sulzberger and Cope [212] state that acne rosacea does not as a rule respond to riboflavin therapy. Johnson and Eckhardt further state that they isolated *Demodex follicularis* from the skin of rosacea patients and that they were able to infect with it the skin of riboflavin deficient rats, but not the skin of normal rats. They believe that the *Demodex* is a secondary invader of skin containing dilated capillaries.

Johnson and Eckhardt [98, 180, 192] believe that rosacea keratitis is of nutritional origin. They noted that the diet of patients with this condition is low in milk, liver and eggs—good sources of riboflavin—and that many of them show some degree of riboflavin deficiency as judged by retention tests. Patients with rosacea retained 47·5 per cent. of a dose of 5 mg. given intramuscularly, compared with normal controls who retained 21·5 per cent. The validity of these tests is open to doubt. Connors, Eckhardt and Johnson [192] postulate that corneal disease may result from a dietary deficiency of riboflavin or from a disturbance of riboflavin metabolism. They claim to have successfully treated rosacea keratitis,

marginal corneal ulcers and catarrhal corneal infiltrates with 1 mg. riboflavin intravenously daily, supplemented by a vitamin B complex preparation containing 880 μ g of riboflavin per ounce, one ounce being taken orally three times a day.

As Wise [270] points out many factors, including riboflavin deficiency, which have been suggested as aetiological in rosacea keratitis, are probably not fundamental, although they may be contributory. He administered large doses of riboflavin to twenty-one patients with the condition, but failed to cure the eye lesions. Spontaneous remissions often occurred without any change in diet. Fish [271, 298] states that rosacea keratitis may occur without riboflavin deficiency and is not cured by treatment with it. A series of forty-five cases all of whom had cutaneous lesions as well failed to benefit from riboflavin therapy. Many of them became worse owing to the withdrawal of atropine during the test. Fish considers that neither acne rosacea nor rosacea keratitis are manifestations of ariboflavinosis and that most symptoms are due to secondary infection, which can be controlled by sulpha drugs such as sulphathiazole, sulphadiazine and sulphacetamide. She points out that the type of corneal vascularization seen in ariboflavinosis is not the same as that present in rosacea keratitis.

Grimsdale [95] has used riboflavin with apparently beneficial results in the treatment of corneal ulcers, photophobia and non-infective conjunctivitis, without any typical signs of ariboflavinosis. Rones and McKay [181] gave riboflavin to twelve patients with diverse chronic corneal lesions and without signs of riboflavin deficiency. The cases, which included phlyctenular keratoconjunctivitis, corneal ulceration, superficial punctate keratitis and sclerosing keratitis were all stated to benefit from the oral administration of 5 to 10 mg. of riboflavin daily, even cases which had not responded to previous local therapy.

Sydenstricker and his colleagues [91, 115] have observed several cases of syphilitic keratitis in which treatment with riboflavin was followed by rapid improvement during periods in which antisymphilitic treatment was not given. This has been confirmed by Gosgrove and Day [137] and Clark [140], although Wagener [100] failed to obtain any improvement. Kruse and Sydenstricker [91, 92] discuss the relationship of syphilis to ariboflavinosis and keratitis, and raise the question whether syphilis produces keratitis only when the nutrition of the cornea is impaired, as in ariboflavinosis.

Castellanos [276], accepting the erroneous assumption that strong light inactivates the riboflavin of the eye (p. 818), states that vernal conjunctivitis is caused by ariboflavinosis. Of one hundred and five patients treated with local anaesthetics and riboflavin 1 to 8 mg. daily, he claims that ninety-two per cent. showed immediate improvement. As the work was not controlled and vernal conjunctivitis is a persistently recurrent condition further proof is required of any association between it and ariboflavinosis.

Claims have been made that treatment with riboflavin is effective in a number of other clinical conditions, but the reports need confirmation. Schwartzman, Dragutsky and Rook [97] report the successful treatment

with riboflavin of a case of Ritter's disease (dermatitis exfoliativa infantum) the essential lesion of which is a generalized inflammation of the skin followed by suppuration and necrosis resembling pemphigus. Riboflavin in doses of 2 mg. three times a day was given and the skin dressed with fifty per cent. cod liver oil in paraffin. Rapid improvement and cure in eighteen days were reported. Further observations are needed to establish the validity of this report. Topping and Knoefel [99], on a purely experimental basis, claim to have treated a case of pemphigus with riboflavin after all other treatment failed, but Wolf and Lewis [76] failed to observe any response in a case treated with 100 mg. of riboflavin daily. Vorhaus and his co-workers [272] report complete healing in five cases out of six of decubital ulceration treated with 5 mg. of riboflavin daily. Complete healing occurred in from seven to thirty-four days without any other treatment. Riboflavin is stated to be effective in the treatment of some of the manifestations of sprue and coeliac disease [71, 78, 79].

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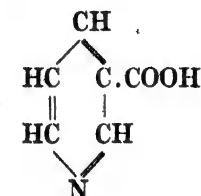
CHAPTER V

NICOTINIC ACID

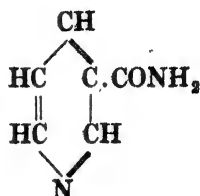
NIACIN

HISTORY

NICOTINIC acid, a member of the B complex, is β -pyridine carboxylic acid. The amide, nicotinamide, is a component of a complex enzyme system.



Nicotinic acid



Nicotinamide

For several years the significance of nicotinic acid in nutrition remained undiscovered. Although it was found in yeast concentrates nearly thirty years ago, little interest was taken in the physiological rôle of pyridine derivatives until 1938, when Warburg and his colleagues showed that nicotinamide is the active group of the coenzyme now known as codehydrogenase II (p. 881). At the same time Kühn and Vetter [2] isolated nicotinamide from heart muscle, and others obtained it from cozymase. As a result of this work considerable interest centred around nicotinic acid as a factor in nutrition. Thus Frost and Elvehjem [8] observed that it had growth stimulating properties, and others showed that it was an essential growth factor for a number of micro-organisms.

A disease of dogs known as black tongue—characterized by glossitis stomatitis, diarrhoea, and typical skin lesions—bears a close resemblance to human pellagra. During their studies on chick pellagra, Elvehjem [4] and his associates isolated nicotinic acid from liver concentrates that were active in curing the condition. They then tested the effect of nicotinic acid on dogs suffering from black tongue; it was curative. This suggested its therapeutic use in the treatment of human pellagra. The first report of its successful use in this connection was made by Elvehjem, Madden, Strong and Woolley [4] in September, 1937. A number of papers by other investigators confirming this appeared in rapid succession [5-7]. Nicotinic acid was at first hailed as the PP or pellagra preventing factor, but it is now known that pellagra is a multiple deficiency disease and that lack of nicotinic acid is only one of the factors in its causation.

As the terms "nicotinic acid" and "nicotinic acid amide" might be unacceptable to the general public, who might consider them to be poisonous like nicotine; the Food and Nutrition Board of the U.S.A. National Research Council suggested in 1942 that the terms "niacin"

and "niacin amide" be accepted as synonymous with "nicotinic acid" and "nicotinic acid amide" respectively [156]. The Food and Nutrition Board suggest that the original names be retained in scientific literature.

CHEMISTRY OF NICOTINIC ACID

Nicotinic acid is a white crystalline solid, melting at 228°–229° C., soluble in hot water and alcohol. It is not only one of the most thermo-

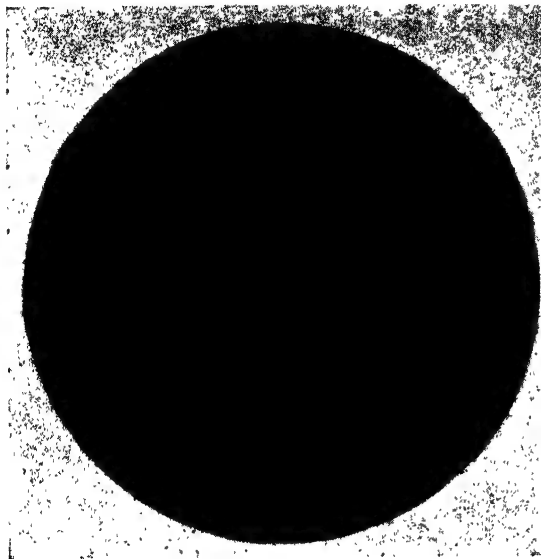


FIG. 79. Nicotinic Acid Crystals.

stable components of the vitamin B complex, but it is one of the most stable of the vitamins, and is not oxidized or destroyed in the ordinary processes of cooking, or by exposure to air, light or alkalis. It can be made bacteriologically sterile by autoclaving without loss of potency. Being an acid it readily forms salts and an amide, which are also physiologically active.

Nicotinic acid has been estimated in body fluids and foodstuffs by a variety of methods. One chemical method depends upon the colour

produced with cyanogen bromide and a primary or secondary amine, such as aniline [8], metol [9], *p*-aminoacetophenone [11], *m*-phenylenediamine [262] and orthoform [16]. It is stated that the *p*-aminoacetophenone method, developed by Harris and Raymond [11] is the most suitable. Another method depends on the formation of a coloration when the dry material under test is fused with 2 : 4-dinitrochlorobenzene [18]. None of these methods is specific as the same colours are given by other pyridine derivatives. Biological methods have been devised such as the cure of black tongue in dogs [14], and the *Lactobacillus* test [12], in which the amount of lactic acid produced by *Lactobacillus arabinosus* is proportional to the nicotinic acid content of the medium. Another method depends on the use of bacterial enzymes present in bacteria which have been adapted to grow on nicotinic acid [155]. A micromanometric method for estimating nicotinic acid combined as diphosphopyridine nucleotide (codehydrogenase I, p. 881) has been devised. It depends on the liberation and measurement of carbon dioxide during a reaction catalysed by diphosphopyridine nucleotide.

UNITS OF NICOTINIC ACID

Since nicotinic acid was known as a pure chemical substance long before it was identified as a vitamin, there has never been any necessity for a biologically standardized unit.

DISTRIBUTION IN FOODSTUFFS

Nicotinic acid occurs in all living cells. Liver, the adrenals, kidney, yeast, whole grain products, flesh foods ("meat"), mushrooms, and peanuts are among the best sources. Denatured cereals, such as white flour or polished rice, fruit and vegetables in general and milk are poor sources. Meat extracts contain appreciable quantities, *e.g.*, a teaspoonful, such as is used to prepare a drink, may contain 10 mg. [164]. Free nicotinic acid is not found in living cells, but as the amide or as part of complex enzyme systems in which it is chemically bound (p. 381).

Since nicotinic acid is heat stable and resistant to oxidation and the action of light, very little is lost in food during cooking and processing. Any losses that do occur result from the vitamin diffusing into the cooking water, which is usually thrown away. In the cooking of meat eighty to eighty-five per cent. is retained after roasting, seventy-seven per cent. after frying, and sixty-five per cent. after braising; the total retention in the meat and drippings in domestic cooking averages seventy per cent. and may be as high as ninety per cent. [165, 166]. In the curing of meat some eighty-four per cent. is retained [166]. Food cooked in cafeterias and restaurants usually contains less nicotinic acid than food cooked at home. Losses up to sixty per cent. have been recorded [167]. In the cooking of vegetables the loss of nicotinic acid is from eight to twenty-two per cent.; the cooking water contains twelve per cent. on an average (range two to forty per cent.) [170]. In the dehydration and canning of meat products eighty-eight to ninety-four per cent. of the nicotinic acid is retained [263].

Nicotinic Acid Content of Foods [168-171]

Food.	Description.	Nicotinic Acid in micrograms (μ g) per gram.
Cereal Foods		
Barley	Red	47
"	Pearl	27.5
Bread, wheat	White	6.6-10.0
	Wholemeal	37-42
	National bread (1948) . .	19.6
Buckwheat	—	44
Corn	—	15.6-26.0
"	Meal	17.6
"	Flour	3
Macaroni	—	21
Millet	—	8
Oatmeal	—	6-11
Oats	—	11.3-16.0

Nicotinic Acid Content of Foods—continued.

Food.	Description.	Nicotinic Acid in micrograms (μ g) per gram.
Cereal Foods—continued		
Rice	Polished	9.0
	Unpolished	44-66
	Milled	16
	Bran	300
	Polishings	284-1,400
Rye	Flour, whole	12.2
	„ bleached	7.3
Semolina	—	20
Spaghetti	—	21
Tapioca	Boiled	3
Wheat	Whole	28-41 ; 54-80
	Flour, white	6-9
	National flour—	
	85 per cent. extraction (1948)	17-20
	82½ per cent. extraction (1944)	18
	Germ	84-70
	Bran	300-850
	Middlings	92-177
	Screenings	192
Proprietary Cereal Products		
[264]		
All Bran	Kellogg's	160-185
Bemax	—	60
Cerevim	Kederle (Vitamin concentrate added)	203
Corn Flakes	Kellogg's (Vitamin concentrate added)	16
„ „	Post's (Vitamin concentrate added)	18
Cream of Rice	—	17
Cream of Wheat	Vitamin concentrate added	16-20
Force	—	41
Grape Nuts	Post's (Vitamin concentrate added)	39-49
Oats	Quaker	10
Rice Krispies	Kellogg's (Vitamin concentrate added)	80
Shredded Wheat	Kellogg's	45
Soya Wheat	—	34
Vegetables and Vegetable Products		
Asparagus	—	11
Beans	Green	3.5-7.6
	Lima	4
Beets	Root	4-6.4
	Greens	3.0
Broccoli	Leaves	14.4
Brussel sprouts	—	4
Cabbage	—	1.2-4

Nicotinic Acid Content of Foods—continued

Food.	Description.	Nicotinic Acid in micrograms (μ g) per gram.
<i>Vegetable and Vegetable Products—continued</i>		
Carrot	—	4; 14.7
Cauliflower	—	4.8-6.6
Celery	—	1.8-2.6
Cucumber	—	1.00-3.2
Egg plant	—	6
Endive	—	7.2
Kohlrabi	—	2.7
Lettuce	—	2-5
Mushrooms	—	69
Onion	—	1.0-5
Peas	Green	7-18
Peppers	Green	2.0
Potatoes	White	11.8
	Sweet	12.9
Pumpkin	—	7
Radish	—	1.5-24
Soya bean	Flour	7.2-24
" "	Bread	13.5
Spinach	—	5-7.2
Squash	—	9.6
Tomatoes	Whole	5.8
	Juice	1.0
Turnip	—	6.9
<i>Beans and Nuts</i>		
Almond	—	11.7
Bean	Kidney, dried	17.1-28
"	Lima, dried	12.7-18.3
	Broad, dried	21
Coconut	—	18.2
Lentils	Dried	31
Peas	Dried	18-28
Peanuts	Raw	86
Peanut butter	—	186
<i>Fruits</i>		
Apples	—	0.9-5
Banana	—	3-6.1
Cherries	—	1.4
Cranberries	—	12.9
Dates	—	8; 21.8
Figs	Fresh	6.3
	Dried	17.2
Grapes	—	8.4
Grape-fruit	Juice	1.5-2.1
Lemons	—	1.5-1.9
Limes	—	1.0-2.7
Orange	—	2.2
Peaches	—	3-9.5
Pears	—	0.9-2.0
Plums	—	1.18-5.6
Pineapples	—	1.86

Nicotinic Acid Content of Foods—continued

Food.	Description.	Nicotinic Acid in micrograms (μ g) per gram.
Fruits—continued		
Prunes	—	4.0
Raisins	—	2.9-6.3
Strawberries	—	2.3-2.6
Dairy Products		
Cheese	Cheddar	2
Eggs	White	0.76
	Yolk	0.35
	Dried	2-6
Milk	Cows', fresh	0.8-1.0
	„ condensed	1.8
	„ skimmed, powdered	6-8.9
	Human	2.6
Meat and Meat Products		
Beef, fresh	Brain	35-49
	Heart	76-84
	Kidney	73.4
	Liver	120-179
	Muscle	46-63.9
	Tongue	71
	Pancreas	58.4
Beef	Extract	375-1,025
„	“ Corned ”	24-95
Chicken	Breast	151
	Muscle, leg	72
	Liver	80-152
Mutton and Lamb	Muscle	45-77
	Brain	32
	Heart	60
	Kidney	60
	Liver	176
Pork	Brain	64
	Heart	73
	Kidney	98
	Liver	140-228
	Muscle	61
	Bacon	44
	Ham	88
	Loin	88
	Smoked ham	82
Rabbit	Brain	12
	Muscle	65-126
	Liver	143-220
Veal	Heart	106
	Liver	170
	Muscle	65-170
Fish		
Cod	Flesh	28
	Liver	16
	Roe	15.2
Crab	—	28

Nicotinic Acid Content of Foods—continued

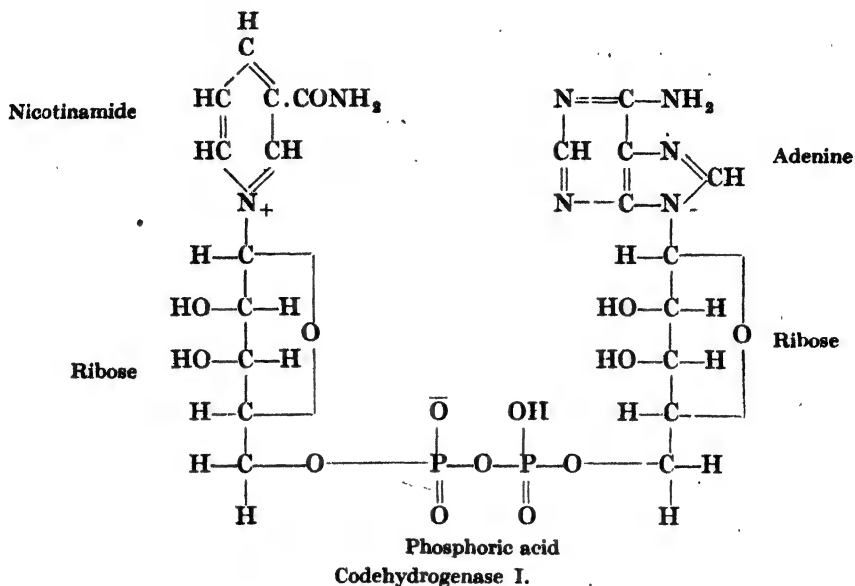
Food.	Description.	Nicotinic Acid in micrograms (μ g) per gram.
<i>Fish—continued</i>		
Haddock	—	9
Halibut	—	30-40
Herring	Milt	23-6
	Roe	23-6
	Flesh	29-40
Mackerel	—	55-72
Oyster	—	13
Salmon	Fresh	84
	Tinned	60
Trout	—	35
Turbot	Muscle	23
<i>Miscellaneous</i>		
"Bemax"	—	60
Chocolate	—	11
Malt extract	—	75-184
"Marmite"	—	640-655
Meat extract	—	2,000
Molasses	—	39
Royal jelly	—	50
Yeast	Brewers'	300-1,000
	Bakers'	400-500
	<i>Torulopsis utilis</i> (Food yeast)	400-450

THE PHYSIOLOGY OF NICOTINIC ACID

Nicotinic Acid and Enzyme Systems. Nicotinic acid, like riboflavin, forms part of complex enzyme systems concerned with hydrogen transport in the living cell. The enzymes consist of an apoenzyme and a coenzyme. The apoenzyme is a specific protein, believed to have no enzyme action itself, which is linked to the coenzyme, the prosthetic group of the enzyme system. The coenzyme is probably the specific substrate concerned with hydrogen transport. There are two coenzymes associated with hydrogen transporting enzymes (dehydrogenases) known as co-dehydrogenases I and II, formerly known as coenzymes I and II.

Codehydrogenase I (p. 382) is identical with diphosphopyridine nucleotide, a complex of one molecule of nicotinic acid amide (nicotinamide), one of adenine, two of ribose and two of phosphoric acid [17, 18].

Codehydrogenase II contains three molecules of phosphoric acid (triphosphopyridine nucleotide). It is structurally similar to codehydrogenase I with an additional molecule of phosphoric acid. Both occur in animal and plant cells, although codehydrogenase I appears to be present to a greater extent. All living cells can synthesize the codehydrogenases from nicotinic acid. Yeast and red blood cells [20] contain relatively large amounts of both. Fresh yeast contains about 500 micrograms per gram, and human muscle 100 to 400 micrograms per gram. Synthesis of



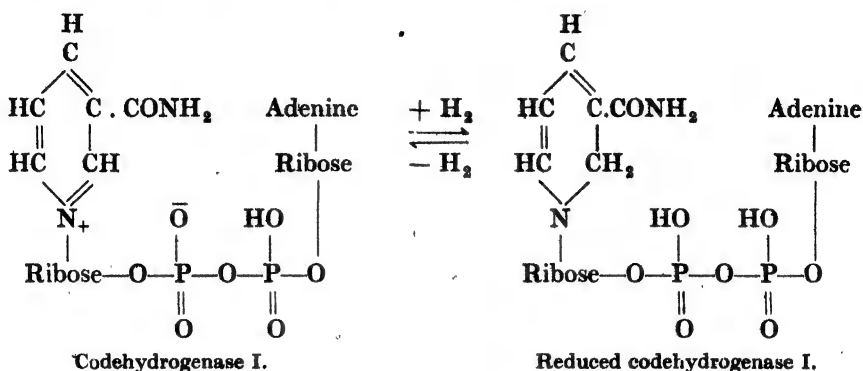
codehydrogenase from nicotinic acid probably occurs in both the nucleated blood cells [157] and the erythrocytes [48] of the blood. In uncomplicated nicotinic acid deficiency there is a decrease in the codehydrogenase I content of liver and muscle [190].

The function of the codehydrogenases is to catalyze the dehydrogenation of various substrates. The following table gives some of the dehydrogenation reactions in which they are involved [158]:—

Substrate and Dehydrogenation Product.	Source of Apoenzyme.	Codehydrogenase.
Lactic acid \rightleftharpoons pyruvic acid	Heart muscle	I
Alcohol \rightleftharpoons acetaldehyde	Yeast	I
Malic acid \rightleftharpoons oxalacetic acid	—
Triosephosphate \rightleftharpoons phosphoglyceric acid	Skeletal and cardiac muscle	I
$2R.CHO + H_2O \rightarrow R.CO_2H + R.CH_2OH$ (aldehyde mutation).	Liver	I
Formic acid $\rightleftharpoons CO_2 + H_2O$	Seeds and <i>E. coli</i>	I
β -hydroxybutyric acid \rightleftharpoons acetoacetic acid	Heart muscle	I
Glucose-6-monophosphate \rightarrow phospho- hexonic acid (Robison's ester).	Yeast, erythrocytes	II
Decarboxylation and dehydrogenation	Yeast	II
Glucose \rightleftharpoons gluconic acid	Liver, yeast	I or II
Citric acid \rightleftharpoons α -ketoglutaric acid	Liver, yeast	II

During the dehydrogenation the codehydrogenase absorbs two atoms of hydrogen from the substrate to form a dihydro-compound. This in turn gives up its two atoms of hydrogen to molecular oxygen, i.e., it is oxidized, and codehydrogenase is reformed. The codehydrogenases thus undergo a reversible reduction-oxidation process, the nicotinamide part

of the molecule being involved in the change. It is believed that upon reduction the nitrogen of the pyridine ring is reduced from the quinquevalent to the tervalent condition.



Codehydrogenase I and II are coenzymes involved in phosphorylation and pyruvic acid oxidation. They form part of an oxidation-reduction system with the flavoproteins (p. 316).

The content of codehydrogenase I and II in the blood can be increased, *e.g.*, by eighty-five per cent., by the administration of excessive quantities of nicotinic acid; the increase varies with the amount of nicotinic acid administered [20, 21]. It has been suggested that the determination of codehydrogenase I in blood might be used as a test for human nicotinic acid deficiency. It is, however, of no diagnostic value since the blood level of codehydrogenase I in pellagrins may fall within the normal range, although in many cases it is lowered [20]. After nicotinic acid therapy the level of codehydrogenase I and II in the blood of pellagrins is raised [22]. Axelrod [23] and his associates determined the codehydrogenase I content of striated muscle in normal subjects and in pellagrins and they found that it decreases as the pellagra becomes more severe.

It has been shown that a number of pyridine derivatives related chemically to nicotinic acid have a nicotinic-like action and can replace it in the codehydrogenases. These derivatives include the salts of nicotinic acid, nicotinamide, the N-diethylamide of nicotinic acid (nikethamide B.P.), ethyl nicotinate and other nicotinic acid esters [287], nicotinuric acid, nicotinamide-glucosido-iodide, quinolinic acid, pyrazine 2:3-dicarboxylic acid and pyrazine monocarboxylic acid. Nicotinamide, nikethamide, quinolinic acid, 2:6-dimethyl pyridine-3:5 dicarboxylic acid and pyrazine mono- and 2:3 di-carboxylic acids have been found to be effective in the treatment of pellagra in place of nicotinic acid [57-60]. Not only are these compounds active in doses comparable with that of nicotinic acid (up to 1,000 mg.), but they also cause an increase in the concentration of codehydrogenases I and II in the blood. It is assumed that these compounds are converted in the body into nicotinamide. The nitrogen atom in the pyridine ring must apparently be unsubstituted for the compound to show nicotinic acid activity. Thus trigonelline (p. 389), in which the nitrogen is methylated, is inactive. This is understandable as in the

codehydrogenases the nitrogen atom of the pyridine ring links up with the ribose moiety of the molecule ; in trigonelline it is blocked.

It has been assumed by Stannus [79] that the manifestations of nicotinic acid deficiency, including some of those of pellagra, result from the failure of those respiratory mechanisms involving the codehydrogenases. Death in canine black tongue, however, is not due to a deficiency of these coenzymes with a consequent failure of tissue respiration [172]. This observation does not necessarily prove that nicotinic acid in the codehydrogenases does not play an important part in tissue respiration.

It is known that sulphonamides such as sulphapyridine and sulphathiazole prevent bacterial growth by interfering with the functioning of chemically related enzyme systems (p. 120). West and Coburn [178] noted the chemical similarity between sulphapyridine and nicotinamide—both have a pyridine ring—and reported *in vitro* experiments with *Staphylococcus aureus* on the basis of which they suggested that sulphapyridine exerts its bacteriostatic effect by interfering with the formation of coenzymes from nicotinamide. The coenzyme systems are essential for cell respiration in micro-organisms. This view has been confirmed [174]. Apparently sulphapyridine inhibits the action of nicotinic acid by preventing the formation of the coenzyme systems in which nicotinic acid participates [184]. It does not affect the activity of preformed coenzymes [175]. Sulphapyridine not only blocks nicotinic acid in the nutrition of micro-organisms, but it inhibits its curative effect in canine black tongue [86]. Other sulphonamides such as sulphadiazine, sulphanilamide, sulphapyrazine and sulphaguanidine, are unable to block the nicotinic acid coenzyme systems, presumably because they differ structurally from nicotinic acid in having no pyridine ring [179].

A possible relationship between nicotinic acid and the metabolism of sulphhydryl compounds is suggested by the presence of a low cystine content of the finger-nails of pellagrins, unaccompanied by a change in the total protein content, during the prevalence of a florid dermatitis. With the subsidence of the skin symptoms and clinical improvement the cystine content of the finger-nails returns to normal [85].

Nicotinic Acid and Porphyrin Metabolism. It has been suggested that nicotinic acid is associated with porphyrin metabolism, since many of the manifestations seen in pellagra, such as abdominal distress, diarrhoea, pigmentation of the skin, and photo-sensitivity are often found in patients exhibiting acute toxic porphyrinuria [24]. It has been stated that the porphyrin output in pellagra is approximately related to the severity of the skin and mucous membrane lesions and that the excretion returns to normal on a diet rich in yeast and liver and with the regression of the disease [25]. However, porphyrinuria is an inconstant finding in pellagra [26, 27], and appears to be of no diagnostic aid in that disease. Rosenblum and Jolliffe [26] have found that the porphyrinuria in pellagra may decrease without the administration of nicotinic acid, or increase with the administration of nicotinic acid, and while the manifestations of pellagra are regressing. Kühnau [81, 82] has been unable to confirm any constant relationship between nicotinic acid and porphyrinuria ; he observed a lowering of urinary porphyrin in some cases receiving nicotinic acid, but

not in others. Liver dysfunction, which is nearly always present in pellagrins, may explain the porphyrinuria of pellagra, either in the form of a disturbance in hæmoglobin breakdown and the production of excessive coproporphyrin, or of the inability of the liver to excrete porphyrin in the bile. Porphyrinuria in old persons has been observed to disappear after the administration of nicotinic acid [176]. This may have been due to improvement of impaired liver function.

Watson [29] has shown that the colour reaction in the urine of pellagrins, frequently mistaken for that of porphyrin, is in reality due to the pigment urorosein. This is present in the urine of many individuals and is not related to nicotinic acid deficiency or pellagra [80, 177]. Watson states that the administration of nicotinic acid does not affect the production of the pigment, which he believes is, or at any rate contains, indirubin.

Nicotinic Acid and Carbohydrate Metabolism. It has been suggested that there is some relationship between nicotinic acid and carbohydrate metabolism. Sydenstricker [38] has noted glossitis, cheilosis and stomatitis in diabetics that cleared up with nicotinic acid. It has also been observed that the amount of the codehydrogenases in the blood of diabetics is much lower than normal.

Pellagrins show a hypersensitivity to insulin. They are stated to get symptoms of hypoglycæmia more readily than normal individuals after an injection of insulin. This hypersensitivity persists in patients who have lost all clinical signs of pellagra. Neuwahl [178] noted a well-marked depression in the blood sugar curve of normal subjects after administering nicotinic acid and he claims that in diabetics nicotinic acid potentiates the action of insulin, *i.e.*, the blood sugar level is lower with a given dose of insulin and nicotinic acid than with the same dose of insulin alone. Nicotinamide also produces this effect [28]. A dose of 500 mg. of nicotinamide is stated to produce hypoglycæmia [268].

Spies and his co-workers [37] believe that nicotinic acid plays an important part in cerebral carbohydrate metabolism. It is known that the energy of the central nervous system is derived entirely from the combustion of carbohydrate. The liberation of energy occurs not in one large burst, but stepwise by means of several enzyme systems, one of which contains nicotinic acid as a component. A break in the chain of carbohydrate oxidation in the central nervous system may explain some of the mental changes seen in pellagra and nicotinic acid deficiency (pp. 404, 417).

Nicotinic Acid and the Hæmatopoietic System. In the rat the reticulocyte count rises, falls, and then rises markedly seventy-two to ninety-six hours after the intramuscular injection of nicotinic acid. The first rise may be due to a mobilization of reticulocytes, but the second is probably a genuine response to hæmatopoietic stimulation [88]. Small doses of nicotinamide given intramuscularly increase the erythrocyte count in rabbits and guinea-pigs as well. The administration of nicotinic acid (8 mg. per kg.) to rabbits rendered anæmic by bleeding causes a more rapid return of the hæmoglobin content and the red cell count to normal than in controls [189]. According to Hoagland and his co-workers [180]

nicotinic acid causes a marked acceleration of the rate at which erythrocytes oxidise lactate and malate.

Dogs fed diets deficient in nicotinic acid develop a profound macrocytic anaemia. On giving nicotinic acid a reticulocyte response occurs and an elevation of the red cell count [267]. In nicotinic acid deficiency the respiration of erythrocytes is probably interfered with by lack of the codehydrogenases.

Physiology of Plants and Micro-organisms. Nicotinic acid in the form of its amide is a cell constituent of plants and a growth factor for many micro-organisms. Certain micro-organisms can apparently synthesize their own nicotinic acid, others such as *Staphylococcus aureus*, *B. dysenteriae*, *B. proteus*, *C. diphtheriae*, lactic acid bacteria and *Salmonella* and the Flexner bacillus require an external supply [140, 181, 185]. The rat and sheep appear to be able to synthesize their own nicotinic acid [42, 43]. Some organisms not only require nicotinic acid, but nicotinic acid in the form of codehydrogenases. *B. influenzae* is included in this group. Its rate of growth has been used for the estimation of codehydrogenases I and II, which are together known as factor V.

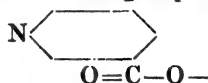
Human Biosynthesis of Nicotinic Acid. It has been previously mentioned that there is strong evidence that bacterial synthesis of vitamin B₁ and riboflavin occurs in the human gut (pp. 186, 317). Recently it has been shown by Ellinger, Coulson and Benesch [265] that nicotinic acid is also probably synthesised by the bacterial flora in the intestine of man. They administered sulphaguanidine and succinylsulphathiazole, which are bacteriostatic in the intestine, to mental patients, and observed a drop in the urinary excretion of nicotinic acid. This would suggest that normally a synthesis of and release of nicotinic acid by intestinal flora takes place and that this synthesis is prevented by the bacteriostatic action of the sulpha drugs. According to Ellinger and Coulson [266] in man the excretion of nicotinic acid may exceed the intake and the amount of nicotinic acid synthesised by the bacterial flora may be as much as eighty per cent. of the daily consumption.

It is possible that the confusional and mental symptoms and the pellagroid rashes occasionally reported after treatment with sulpha drugs may well be due to conditioned nicotinic acid deficiency, following the bacteriostatic action of these drugs on the intestinal flora. It is recommended that when sulphonamides are given to sterilize the gut supplements of nicotinic acid and other vitamins should be given.

Pharmacology. The toxicity of nicotinic acid has been studied by Unna [56]. Four to five grams of nicotinic acid per kilo of body weight are necessary to produce acutely toxic effects in mice and rats; the amide is twice as toxic in these animals, although in human beings the amide is better tolerated than the acid itself. Nicotinic acid is not toxic to rats, chickens and dogs if taken over a prolonged period (two months) in doses of 2 grams per kilo of body weight. Toxic doses result in ataxia and cyanosis. Bean and Spies have given it to over 1,000 persons without any toxic effects resulting.

Nicotinic acid possesses a pronounced vasodilator action, which was observed by the earlier workers when they used it in the treatment of

pellagra [61-68]. The effects noted both in pellagrins and in normal persons include flushing of the face and neck, a sensation of heat, tingling and itching, which come on within seven to ten minutes and last about thirty minutes. There is slight dizziness, thumping in the head, headache, and sometimes nausea, vomiting and transient abdominal pain; the blood pressure is not appreciably affected. An urticarial rash, palpitations, cyanosis of the nails and mental depression have also been described. Reddening and flushing of the skin may occur with either an increase or a decrease in the skin temperature. The symptoms are only transitory and are not harmful, although disturbing to the patient. Sebrell and Butler [61] include gastro-intestinal symptoms, substernal oppression, and pruritis among the symptoms. Oral doses of 100 to 300 mg. or 20-25 mg. intravenously will cause an increase of skin temperature. Bean and Spies [64] have shown that this response is also given by the sodium, ammonium, and monoethanolamine salts of nicotinic acid and by its ethyl ester, and by pyrazine monocarboxylic acid, all of which are effective in the treatment of pellagra. We have observed these reactions following exposure to the vapour of methyl nicotinate. The vascular reaction is probably associated with the grouping



This vasodilator action is checked by glycine in doses of 30 to 60 gm. and by adrenaline [182].

Field and Robinson [65] have shown that these vasodilator reactions are not produced by nicotinamide, the vitamin activity of which is equal to that of nicotinic acid itself. They saturated patients with 500 mg. doses of nicotinic acid until flushing occurred following 40 mg. doses of the acid. The administration of the same dose of nicotinamide was not followed by peripheral vasodilation, or any other symptoms, which follow the ingestion of large doses of nicotinic acid. Nicotinamide may act by preventing the breakdown of codehydrogenase I [66].

Loman [187] and his colleagues have shown that nicotinic acid is a peripheral vasodilator, and they infer that its vasodilator action is arteriolar. There is no significant change in body temperature or metabolic rate so that the vasodilatation is presumably not compensatory to increased heat production, but probably to a local effect on the arterioles in the skin [182]. The flushing, itching and heat of the skin, increased motility of the stomach and the secretion of gastric hydrochloric acid that occur after administering nicotinic acid are similar to those produced by histamine. It may well be that nicotinic acid favours the release of histamine. Nicotinic acid produces not only vasodilatation of the skin, but also of the brain and spinal cord, as shown by observations on the calibre of cerebral and spinal vessels [126]. In the rabbit hyperæmia of the kidney follows the injection of 10 to 20 mg. of nicotinic acid per kg. of body weight [285].

Aring and his co-workers [141] have shown that nicotinic acid and quinine nicotinate administered intravenously increase the rate of intracranial blood flow in human beings for twenty to sixty minutes, without

any significant change in blood pressure. Since Moore [126] noted an increase in the width of the pial vessels in the cat after the injection of nicotinic acid, presumably these vessels, at least, are involved in the process, which occurs within several minutes of the injection. The dilator effect of nicotinic acid roughly parallels the reaction of the skin. Nicotinic acid derivatives which do not cause flushing of the skin (*e.g.*, nicotinamide) do not increase the rate of intracranial blood flow. Since nicotinamide relieves some of the symptoms of pellagra, it is probable that the relief of certain mental and nervous manifestations of pellagra by nicotinic acid does not depend on the dilator effect of this substance on the intracranial vessels.

It was concluded by Popkin [67] that this vasodilator effect of nicotinic acid was too inconstant and evanescent to be of therapeutic value, but reinvestigation of the problem by means of plethysmographic studies suggest that it may be of value in the treatment of extremities with a diminished blood supply. Abramson [68] found a significant increase in the blood flow to the hand and forearm, although not to the leg, after administering doses of 100–800 mg. of nicotinic acid by mouth or 20 to 25 mg. intravenously. The effect was due to local changes at the periphery, rather than to an increase in cardiac output, since neither the pulse nor the blood pressure were affected. No tolerance was developed during the administration of nicotinic acid over three weeks. Loman [187] has shown that nicotinic acid can cut short a Raynaud attack artificially produced by the injection of adrenaline into the brachial artery.

Nicotinic acid or the sodium salt produces a transient rise of blood pressure when given intravenously to animals. It is claimed that nicotinic acid has blood coagulating properties; it may promote the formation of more prothrombin in the liver [40]. In doses of 500 mg. nicotinic acid is stated to produce polyuria, an increased excretion of organic acids and slight albuminuria [269].

Frankau [188] has shown in a series of carefully controlled experiments, the results of which were submitted to statistical analysis, that the administration of nicotinamide in doses of 50 mg. to 200 mg. to active young men results in increased efficiency in carrying out fairly severe tests involving both physical effort and co-ordination. There was a well-marked diminution in the time taken to complete the test and less fatigue in the subjects receiving nicotinamide compared with controls.

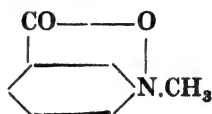
Absorption, Storage and Excretion of Nicotinic Acid. Nicotinic acid is present in foodstuffs mainly as coenzymes. It is not known whether these are absorbed directly as such or hydrolysed to nicotinic acid or nicotinamide first. If nicotinic acid or the amide are given by mouth they are absorbed unchanged. Nicotinic acid is converted into the amide after absorption into the blood stream. In normal persons the blood nicotinic acid ranges from 0.80 to 0.83 mg. per 100 c.c. of blood [45–48, 84]. The greater part is in the blood corpuscles [84] and is present as the coenzymes [186]. Ingestion of nicotinic acid causes a rise in the coenzymes in the blood of both pellagrins and normal persons [22].

Nicotinic acid is present in practically all tissues; the liver contains more than any other organ. There is a direct correlation between the

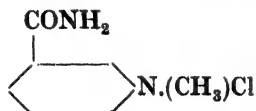
tissue nicotinic acid and the codehydrogenase I content of muscle and liver and the nicotinic acid intake in the diet [190]. In pellagra it is stated that the codehydrogenase I content of striated muscle is lower than normal, but the amount in erythrocytes shows only a slight decrease. A period of months is required to deplete the body of sufficient of its stores of nicotinic acid to produce pellagra [197]. Chronic alcoholics and others with liver damage may have difficulty in storing nicotinic acid [198].

Like other vitamins nicotinic acid is secreted in the milk, and to a lesser extent in the sweat, in which it is present from 20% to 100% per 100 c.c. [187, 188].

Nicotinic acid is excreted as such in the urine and partly as the nicotinamide coenzymes I and II, and as traces of nicotinuric acid. The dog only excretes trigonelline and nicotinuric acid, but no nicotinic acid or nicotinamide. It is generally considered that in man ingested nicotinic acid is also excreted partly as trigonelline,



but this is disputed by Coulson, Ellinger and Holden [282, 283], who state that it is probable that the so-called "trigonelline" is really nicotinamide methochloride,



and that the trigonelline actually present is mainly due to that present in the diet. According to Ellinger and Coulson [283], the normal daily excretion of nicotinamide methochloride is about 7.5 mg. with a range of 2 to 8 mg. and represents approximately fifteen per cent. of ingested nicotinamide. This corresponds to an intake of 40 mg. of nicotinamide or nicotinic acid daily. This value is inconceivably high, the average value obtained from dietary surveys being less than 20 mg. (p. 391). This apparent discrepancy may be due to the bacterial synthesis of nicotinic acid or its derivatives in the human gut, which has been demonstrated by Ellinger, Coulson and Benesch [265]. The amount of nicotinamide methochloride eliminated is probably influenced by physical exertion [283].

According to Ellinger and Coulson [283] a rapid increase in the elimination of nicotinamide methochloride occurs after the ingestion of nicotinic acid, nicotinamide, nikethamide and nicotinic acid monoethylamide (*cf.* p. 383). They doubt whether measurement of the excretion of nicotinamide methochloride after a test dose of nicotinamide is of value for the estimation of nicotinic acid deficiency, as the elimination of nicotinamide methochloride depends upon a number of factors, including not only the intake of nicotinic acid and its derivatives, but also exercise, food and the presence of methyl donors (p. 114), and the efficiency of the methylating mechanism in the body.

Subjects with pellagra generally show a lowered nicotinic acid excretion, although Briggs [50] states it does not differ significantly from normal. Spies and his co-workers [51] noted a considerable variation in the nicotinic acid content of urine both in the same individual and from person to person. The amount excreted depends on the diet, the amount of nicotinic acid administered and the mode of administration. Thus a pellagrin retains more of a test dose than a normal person. There is an increase in the excretion of the coenzymes after administering nicotinic acid. An increase in the protein content of the diet causes a decreased excretion of nicotinic acid and *vice versa* [191].

The daily output of nicotinic acid is very variable as the following table shows :—

Daily Excretion of Nicotinic Acid

	Normal.	Level Indicating Deficiency.
	mg.	mg.
Bandier [193]	2.45- 2.50	—
Briggs [50]	0.16- 2.36	0.06-2.89
Ghosh [189]	3.0 - 5.3	1.8 -2.9
Golberg and Thorp [192]	1.5 - 4.5	0.0 -3.7
Harris and Raymond [11]	3.0 - 5.0	1.8 -2.9
Kochhar [194]	3.0 -10.8	—
Kühnau [195]	0.5 - 3.0	0.64-1.05
Melnick, <i>et al.</i> [54]	1.7 -29.3	—
Naganna, <i>et al.</i> [144]	4.8 - 6.1	0.0 -1.8
Patton, <i>et al.</i> [45]	3 - 5	—
Ritsert [8]	1 - 6	—
Rosenblum and Jolliffe [9]	3.4 -10.2	0.0 -2.8
Swaminathan [8, 196]	2.06- 8.88	0.42-2.2
Wang and Kodicek [11]	0.46- 2.33	0

Some workers consider that low excretion levels indicate nicotinic acid deficiency, but it will be seen from the table that the so-called normal and deficiency levels overlap.

Oral administration of test doses of 100 mg. to 300 mg. of nicotinic acid or its amide causes a variable and transitory rise in the urinary excretion, showing a peak at the end of the first hour. Maximum excretion occurs in the first three hours (Wang and Kodicek [11]). The output after ingestion of test doses fluctuates considerably. About 63 per cent. of a dose of 100 mg. is excreted in the first three hours and 90 per cent. in the first five hours.

According to Sarett *et al.* [53] the normal excretion of nicotinic acid is 1-3 mg. daily compared with 30-50 mg. of trigonelline. The excretion of the latter may be considerably increased by smoking or coffee drinking [52, 55]. Thus up to 200 mg. a day may be excreted by coffee drinkers. The daily excretion of nicotinic acid, trigonelline and related substances averages 20-50 mg. daily [53]. In studying trigonelline excretion in man

it is important to exclude coffee, nuts and legumes from the diet. Then the excretion is constant around 9 to 18 mg. a day. Smoking should also be stopped. There is a constant excretion of about 10 mg. of trigonelline daily from endogenous sources that is independent of food intake [142]. Goldsmith [202, 281] estimated the excretion of trigonelline after a test dose of 300 mg. of nicotinamide. It varied from 20 to 45 mg. with an average of 30 mg. in the first six hours. Subjects suffering from diseases such as pellagra and ariboflavinosis excreted from 8 to 35 mg. of trigonelline, with an average of 16 mg. in the first six hours. These views on the excretion of trigonelline are challenged by Coulson and Ellinger [282, 283], who state that the so-called trigonelline is nicotinamide methochloride (p. 389).

HUMAN REQUIREMENTS OF NICOTINIC ACID

It has been found that 0.15 mg. of nicotinic acid per kilogram of body weight per day is sufficient to protect dogs against black tongue [199]. All the available evidence suggests that man has approximately the same nicotinic acid requirement per kilogram as the dog. The various liver fractions are curative of black tongue and pellagra in approximately the same dose per kilogram [200, 201]. If the dog requires 0.15 mg. per kilogram a man of average weight (70 kilograms) would require approximately 10.5 mg. of nicotinic acid daily. This would represent a minimal requirement, *i.e.*, just sufficient to prevent deficiency disease. Some of the earlier figures were based on the amount of nicotinic acid necessary to keep a mild case of pellagra from relapsing, *e.g.*, 40 to 50 mg. a day.

The human requirements of nicotinic acid have also been calculated from dietary studies. Diets of various groups of individuals have been analysed for their nicotinic acid content. Some of the values obtained are given below :—

Author.	Dietary.	Intake of Nicotinic Acid in mg. per day.
Kodicek, 1942 [143]	Average war-time diet in England, 1942	12
	Pellagra preventing	8-10
	Diet containing white bread	0
	Diet containing wholemeal bread	12.3
Grande Covian and Jiménez García, 1943 [203].	Diets during Spanish Civil War, 1937-1939.	12-22
Cheldelin and Williams, 1948 [204].	Average American diet (2,500 cal.)	11
	Average diet with "enriched" bread and flour	17
Winters and Leslie, 1943 [205]	Low income diet	4
Davies, <i>et al.</i> , 1943 [207]	Munition workers in Britain 1943	11-16.5
Winters and Leslie, 1944 [206]	Moderate income diet	10.5-13
	Low income diet	5-6

The Committee on Food and Nutrition of the National Research Council of the United States recommends the following daily allowances of nicotinic acid :—

Daily Requirements of Nicotinic Acid (National Research Council)

	Calories.	Nicotinic Acid mg. per day.
Man (70 kg.)		
Moderately active	8,000	18
Very active	4,500	28
Sedentary	2,500	15
Woman (50 kg.)		
Moderately active	2,500	15
Very active	3,000	18
Sedentary	2,100	12
Pregnancy (latter half) . .	2,500	18
Lactation	3,000	28
Children up to 12 years		
Under 1 year	100 per kg.	4
1- 3 years	1,200	6
4- 6 "	1,600	8
7- 9 "	2,000	10
10-12 "	2,500	12
Children over 12 years		
Girls, 18-15 years	2,800	14
16-20 "	2,400	12
Boys, 18-15 "	3,200	16
16-20 "	3,800	20

It was originally thought that the daily requirements of nicotinic acid were of the order of 25 to 50 mg. daily. Thus in 1940 Elvehjem [69] gave a figure of 25 mg. a day. Such figures were based mainly on studies on pellagrins. Actually a much lower daily intake is sufficient for the maintenance of good health. Pellagra is rarely seen in England, where the average daily intake of nicotinic acid is 12 mg. [148]. From 8 to 10 mg. is stated to protect against pellagra [148]. A good diet, including liberal quantities of meat and whole cereals, can provide 20 to 25 mg. of nicotinic acid a day. The minimal daily intake is probably in the region of 10 mg. This is confirmed by the observation of Sarett *et al.* [142] that there is a daily excretion of 10 mg. of nicotinic acid and its derivatives when fasting over short periods. An intake of 20 mg. daily for a normal adult allows for losses in the cooking and preparation of food. In the case of very active workers and in pregnancy, with its enormous drain on maternal nutrition, this figure should be increased to approximately 25 mg. daily. The daily allowances of nicotinic acid recommended by the National Research Council of the United States cover the requirements of all types of individuals and can be met by a good diet of natural foodstuffs.

These figures for the human requirements of nicotinic acid may need revision in the light of the recent observation of Ellinger, Coulson and Benesch [265] that bacterial synthesis of nicotinic acid may occur in the human intestine (p. 886). To what extent this occurs and to what extent the nicotinic acid that is synthesized is absorbed is not known.

DISEASES ASSOCIATED WITH DEFICIENCY OF NICOTINIC ACID PELLAGRA

History. The word pellagra, which is a corruption of the Italian *pelle agra*, meaning rough skin, was first used in 1771 by Frapolli, who insisted that the skin lesions of the disease were precipitated by exposure to sunlight. The first comprehensive study of pellagra was made in 1780 by Gaspar Casal, a physician of Oviedo, whose name is commemorated in the lesion of the neck known as Casal's necklace (p. 399). The disease was first recognised as a clinical entity in Spain and Italy but it was soon observed in many other European countries. In 1864 it was first described in North America, although it was undoubtedly widespread before that time. In both Italy and America pellagra became a national menace. In 1881 there were 100,000 cases in Italy with a population of 16,500,000; in 1880 five per cent. of the population were said to be afflicted. The modern history of pellagra starts in 1914 when Funk postulated that pellagra was a food deficiency disease. This was confirmed by the classical investigations of Goldberger and his associates in the years 1918-15. Attempts to transmit pellagra by inoculation of healthy persons with the blood, secretions and preparations made from pellagrous lesions failed, thus showing that the disease was not infectious. After the discovery in 1937 that nicotinic acid cured black tongue in dogs [7]—a disease showing some resemblance to human pellagra—it was used in the treatment of the latter, with good results. It soon became apparent, however, that nicotinic acid, although it relieved some symptoms of the disease was not curative, Liver and a diet rich in the vitamin B complex were found to be more effective.

Distribution of Pellagra. Pellagra, which is endemic in maize-eating areas, occurs among practically all races, chiefly in rural districts where there is a limited choice of food. The disease was once common in Italy, where it was a major problem, but with a rise in the level of nutrition of the population it is now disappearing; less than 100 cases are reported annually, in comparison with numbers running into six figures recorded in the last century.

By far the largest number of cases occur in America chiefly in the Southern States among the negroes. It has been estimated that in 1938 nearly half a million persons in the United States suffered from pellagra and that 3,500 die from it every year [208]. This was an improvement on conditions a decade earlier, when 7,000 died annually in the Southern States, with a death rate of 22.4 per 100,000 of population. The latter figure was reduced to 5.1 in 1940. A considerable improvement occurred after 1929 owing to the free distribution of yeast to sufferers and the growing of crops on small holdings by the rural population. In the Northern States pellagra occurring among the white population is of alcoholic origin. According to Spies [209] one to two per cent. of the admissions to the medical wards of Cincinnati General Hospital, Ohio, U.S.A., suffer from pellagra.

Pellagra is also met with in Spain, Portugal, the Balkans, Greece and

Turkey, South America, India, China, Japan and the Straits Settlements. During and after the Civil War of 1937-39 in Spain there was an alarming increase of all deficiency diseases, particularly pellagra. In Chile there are 3,000 cases annually with a mortality of twenty-six per cent. [210]. Pellagra is rare in Africa, except in Egypt and an area on the east coast. The distribution and incidence of pellagra in warm climates is admirably reviewed by Stannus [75] in a series of papers in the *Tropical Diseases Bulletin*.

Pellagra is rare in Great Britain, although it is certain that a proportion of the cases go unrecognised. Before the nutritional nature of the disease was known it was not uncommon to observe cases in mental hospitals and institutions. It is still met with in psychiatric practice [211] and sporadic cases are occasionally reported [71-73]. Stannus and Gibson [70] review the published cases in the British Isles from 1912 to 1984 which numbered one hundred and thirty-three. Apparently the first entry under *pellagra* in the Annual Report of the Registrar-General appeared in 1912. Davies and McGregor [74] recorded sixteen cases between 1934 and 1939. In 1942 Deeny [188] reported on sixteen cases of pellagra in Northern Ireland, where he says mild forms are relatively common and often pass unrecognised, the patient being diagnosed as suffering from neurasthenia, dyspepsia or eczema. A patient of one of the authors suffering from mild pellagra was treated at a skin hospital for six months for eczema without the true nature of the condition being diagnosed [212].

Ætiology. Pellagra is a multiple deficiency disease. In 1937, when nicotinic acid was found to be of value in the treatment of the condition, it was at once concluded to be due to nicotinic acid deficiency. But nicotinic acid alone does not cure pellagra. Indeed some symptoms of pellagra do not respond to nicotinic acid at all. Moreover, there is no correlation between the incidence of pellagra and the nicotinic acid content of the diet. There were, for example, some inhabitants in Madrid during the Spanish Civil War with a low daily intake of nicotinic acid, yet they did not get pellagra [208]. In any case there is never an uncomplicated nicotinic acid deficiency; if the diet is lacking in nicotinic acid, it is lacking in other factors of the vitamin B complex. Pellagrins commonly suffer from cheilosis, angular stomatitis, retrobulbar neuritis and burning feet, a syndrome associated with riboflavin deficiency (pp. 329, 349). A deficiency of vitamin C is also seen in pellagrins, whose skin lesions are stated to clear more rapidly when treatment includes the administration of vitamin C [78, 218]. Possibly lack of other factors of the vitamin B complex, such as pyridoxine, and a deficiency of vitamin A may play a part in producing some of the lesions of the disease. It is interesting to note that for many years the belief was widely held that since pellagra was endemic in many maize-eating areas, it was caused by a toxin in the grain. This was definitely disproved in 1910 when Stannus [75] described an outbreak of pellagra among African natives on a diet of rice and beans.

The points of similarity between pellagra and pernicious anæmia have been emphasized elsewhere (p. 352). Following Castle's theory of the pathogenesis of pernicious anæmia Stannus [214] and later Harris [80] postulated that deficiency of certain intrinsic (endogenous) and extrinsic

(exogenous) factors might play a part in the causation of pellagra. The intrinsic factor may be located in the liver or stomach [81]; Manson-Bahr [103] suggests that it is the lower part of the small and in the large intestine (ileocolic inefficiency). The extrinsic factor is stated to be present in the food, particularly in liver and yeast and, therefore, may well be one or more members of the vitamin B complex. Nicotinic acid alone, while helpful in treatment, is not curative in pellagra. It is significant that both gastric and liver extracts are of value in the cure of pellagra. Sydenstricker [81] claims that the results obtained with liver extract are far more striking than those obtained with nicotinic acid alone. He has made the interesting observation that liver extract from a dead pellagrins does not

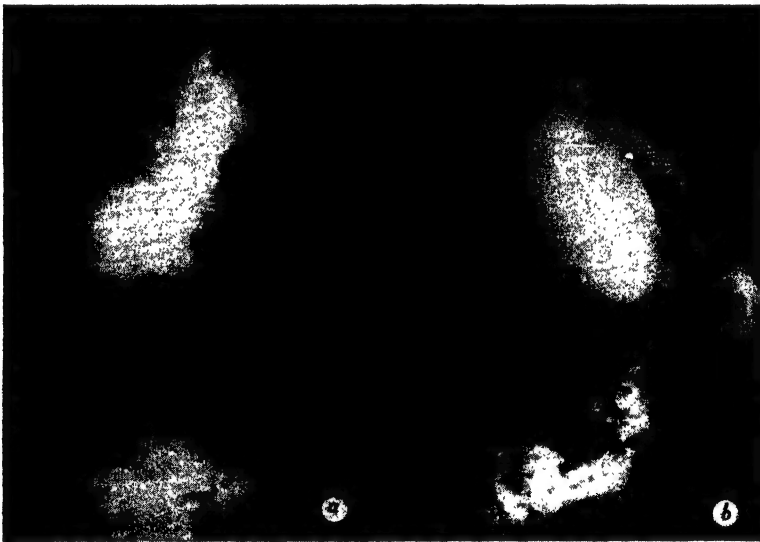


FIG. 80.—“Secondary” Pellagra conditioned by Cardiospasm. Anterior and lateral views of X-ray after swallowing barium meal, showing enormously dilated and tortuous oesophagus with a constriction in the middle third. Same case as Fig. 82.

cure pellagra, whereas that obtained from the liver of a healthy animal does.

There are many external factors that play a part in precipitating an attack of pellagra. They include the following :—

Infestations and Infections. These include malaria, schistosomiasis, amœbic and bacillary dysentery, ancylostomiasis and intestinal tuberculosis. They operate by interfering with the absorption of food (diarrhœa), increasing the general metabolism owing to pyrexia, or by the parasite absorbing foodstuffs from the host.

Increased Metabolism. It is well known that pellagra occurs in women during pregnancy and lactation and among men in prison camps doing forced labour. In females the greatest incidence is in the child-bearing period.

Lesions of the Gastro-intestinal Tract. So-called "secondary" or conditioned pellagra has been recorded following some lesion in or an operation on the gastro-intestinal tract, such as carcinoma of the alimentary tract, œsophageal stricture, gastric and duodenal ulcer, chronic gastritis, enteritis, ulcerative colitis, pyloric stenosis, rectal stricture, short-circuiting operations



FIG. 80. Pellagra. The patient is a labourer wearing trousers but no shirt whilst working in the daytime. A pellagrous dermatitis is present on the part of the arms and body exposed to light. It is absent over the area protected by the patient's braces. Exposure to light may be a precipitating cause of the skin lesions of pellagra.

on the intestine [215], cardiospasm [216, Fig. 80], and gastro-intestinal fistulæ. These operate by interfering with the absorption of the extrinsic factor or by destruction of that part of the bowel producing the intrinsic factor. There is an excellent review by Bean, Spies and Blankenhorn [261] on secondary pellagra.

Alcoholism. Pellagra due to alcoholism is not uncommon in the northern United States. Strong alcohol irritates the gastro-intestinal tract, causes gastritis and secondary infection, which result in faulty absorption of food (*cf.* p. 209). The alcoholic consumes calories but not food. It is also possible that alcohol interferes with the formation of the intrinsic factor in the stomach or the liver.

Restricted Food Intake. The quality and quantity of food consumed may suffer as a result of eating unbalanced diets. Food faddists, eccentrics, asylum inmates, and "slimming patients" have been known to develop pellagra. Pellagrins may become insane (p. 406), but on the other hand the insane may develop pellagra because of nutritional failure. Gastric disturbances and gastritis are also common in psychotics.

Sunlight and Physical Trauma. Exposure to sunlight or any physical trauma may be a precipitating cause of pellagra (Fig. 80). From a study

of 465 cases Ruffin and Smith [85] noted that not only sunlight, but radiation from an electric heater will precipitate not only the dermatitis of pellagra, but also oral and gastro-intestinal symptoms in convalescent patients. This was prevented by liver extract. Sunlight acts as an irritant; exposure to any other form of irritation such as tight clothing, repeated friction, irritating sweat or friction between body surfaces (thighs, nates, scrotum) may cause skin lesions in pellagrins. There is a distinct seasonal incidence, which is higher in the late spring and early summer, due partly to the stronger sunlight and to lack of nutritive foodstuffs in the winter and early spring.

Moore [88] has correlated the distribution of pellagra in Nigeria with the consumption of large quantities of manioc, the glycoside of which he believes inhibits the respiratory enzyme systems. On hydrolysis these glycosides yield hydrogen cyanide which is known to inhibit certain enzyme systems.

Clinical Signs and Symptoms of Pellagra. It is apparent from the study of a number of pellagrins, that there is a long prodromal period of ill-health with insidiously advancing symptoms, which at first appear trivial, but gradually increase in intensity. Loss of weight, strength and appetite, insomnia, vertigo, headache, "dyspepsia," anorexia, sore tongue and mouth, and constipation or diarrhoea are common prodromal symptoms, and appear without obvious cause. In the preclinical stage constipation is common. Irritability, loss of memory, and depression may be complained of. Other early symptoms include abdominal pain, nervousness, palpitation, flight of ideas, inability to concentrate and mental confusion. Shelley [217] states that the earliest symptoms are dizziness on rising, pains in the limbs and vague alimentary symptoms. It is clear that the early syndrome presents no uniform clinical picture, and in the early stages a diagnosis of neurasthenia may easily be made. The diagnosis of preclinical pellagra can be made if these signs and symptoms are associated with grossly inadequate nutrition, with persons suffering from gastro-intestinal disease or who have been submitted to surgery of the gastro-intestinal tract, or with persons whose vitamin requirements have been increased by pregnancy, lactation, infection, diseases of the thyroid, or increased physical exercise (as in prison camps). At this stage the disease is readily arrested by the provision of nicotinic acid and other vitamins in high doses, supplemented by liver, yeast, and an adequate diet.

The facies of pellagra often exists before typical manifestations appear. According to American workers, the pupils are usually dilated (Fig. 91), the sclera is bluish and leaden coloured, the eyes and eyelids move slowly, and there is a characteristic dull lifeless stare (Fig. 81). There is an anxious or querulous expression around the eyes, which is so marked that it may sometimes be of diagnostic aid. The typical pellagrin is profoundly miserable. The ambulant pellagrin has frequently a muddy complexion and a slightly pigmented or macular eruption over the face, particularly the alae of the nose and exposed surfaces of the neck, long before a typical dermatitis appears.

General Symptoms. These are summarized in the mnemonic, "Diarrhoea, Dermatitis and Dementia." The general symptoms include insomnia,

SECONDARY PELLAGRA

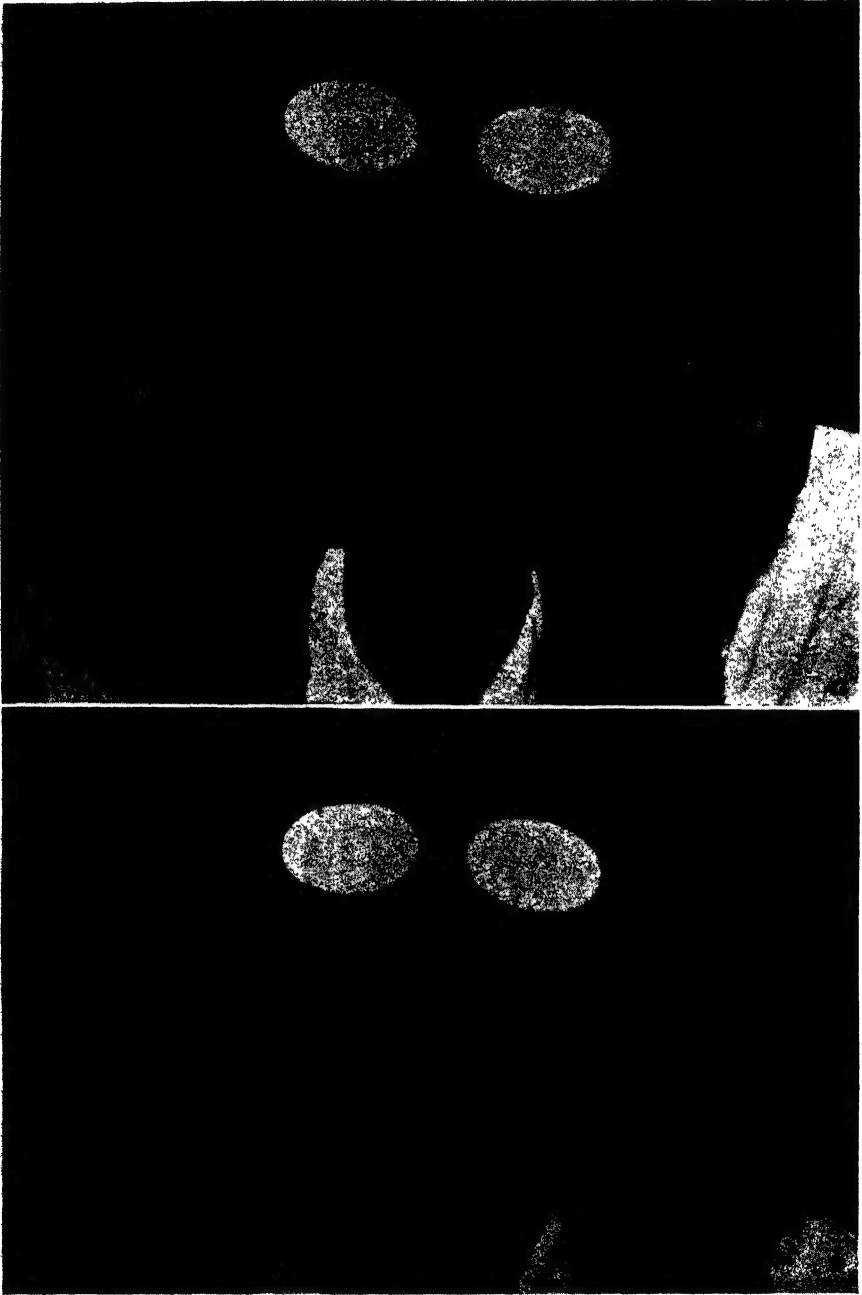


FIG. 82.—“Secondary” Pellagra. The pellagra was due to cardiopasm (see Fig. 80). The upper figure, is before treatment. Note the dermatitis on the back of the hands, neck and nose. The lips are cracked and the tongue red, swollen and beefy looking. The lower figure is after treatment with nicotinamide and high vitamin diet.

loss of weight and strength, vague burning sensations over almost any part of the body, vertigo, staggering and sometimes tinnitus. The head may feel dull, there may be difficulty in concentrating, and cephalgias varying from a sensation of fullness or pressure, localized or general, to boring or stabbing pains are complained of. Any of the prodromal manifestations previously described may be met with. So-called "neurasthenic" symptoms occur in the prodromal stage. More than half of all severe cases have a macrocytic hyperchromic anæmia, and a number show achlorhydria. Pellagra may present no clear-cut clinical picture. The following manifestations are often seen.

Gastro-intestinal Symptoms. These precede the other symptoms and lesions and are usually the presenting ones. Loss of appetite, nausea, indigestion, vomiting, abdominal pain and constipation or diarrhœa are early complaints. Glossitis (Figs 82, 96-98) and stomatitis are among the first symptoms. The tongue is red, first at the tip and edges and later the entire tongue is denuded of superficial epithelium and papillæ (Figs. 97, 98) and is painful and inflamed as in sprue (p. 354). Often there are areas of superficial ulceration over the mucous membranes of the tongue and mouth, and smears from these show large numbers of Vincent's organisms. Dysphagia, a scalding sensation in the mouth, increased by highly seasoned foods or hot drinks, may be so painful that the patient refuses food; vomiting may be serious. There may be a salty, bitter or bad taste in the mouth and the pain on swallowing may cause increased salivation. Œsophagitis is common and food may burn all the way down. Œsophagoscopy under local anæsthesia shows a hyperæmic and œdematous mucosa with multiple small punctate ulcerations, and a barium swallow shows up many small constricted areas along the course of the œsophagus [272]

About fifty per cent. of pellagrins have achlorhydria even after histamine stimulation. In some cases hydrochloric acid reappears in the stomach after treatment with nicotinic acid. Radiological examination of the stomach reveals an atrophic mucosa associated with hypotonia, hypomotility and retarded evacuation [219].

Pellagra often commences with constipation; it is only later that diarrhœa becomes a prominent feature, although it is by no means constant, even in severe pellagra. Chronic diarrhœa when it does occur is distressing, the patient passing anything from three to thirty stools a day. These are liquid, profuse, foul and gaseous; sometimes they resemble those of sprue (p. 355). Frequent defæcation produces a burning feeling in the rectum and proctoscopy shows general inflammation of the mucous membranes. The restricted food intake and the diarrhœa lead to emaciation. Many pellagrins have an irregular temperature with an evening rise up to 101° F. Albuminuria is said to be present in twenty per cent. of the cases [220].

Skin Lesions. Pellagrous dermatitis has a characteristic appearance and is distributed in those parts of the body subject to exposure and mild trauma due to tight clothing. The lesions are precipitated by exposure to sunlight, fires and radiant heat. They are distributed on the face, neck (Casal's necklace), dorsal surfaces of the hands and lower forearms (Figs. 81-87, 90-98), elbows, and the dorsum of the feet and lower legs in bare-

footed persons. There may also be patches over the sternum, scrotum (Fig. 88), labia and anus and other regions subjected to mechanical irritation or the action of the body secretions. These are typical sites, but the dermal lesions may occur on any part of the body. They are usually bilaterally symmetrical and are sharply demarcated from the adjacent healthy skin (Fig. 90), although Bean, Spies and Vilter [271] have described a number of cases of unilateral or asymmetrical pellagrous dermatitis. At

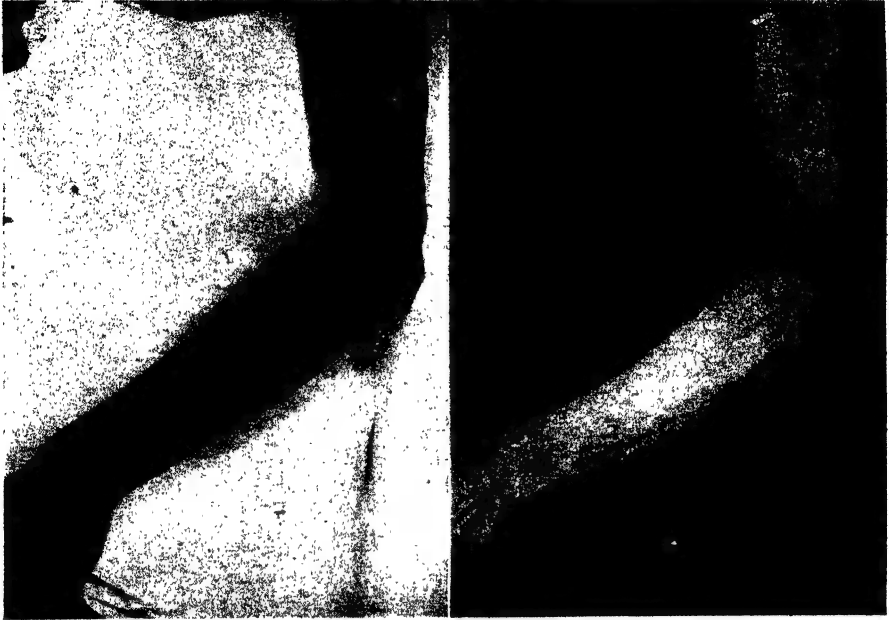


FIG. 88. Pellagrous Dermatitis. The heavily pigmented and cracked condition of the skin is characteristic. A case treated at the London Hospital.

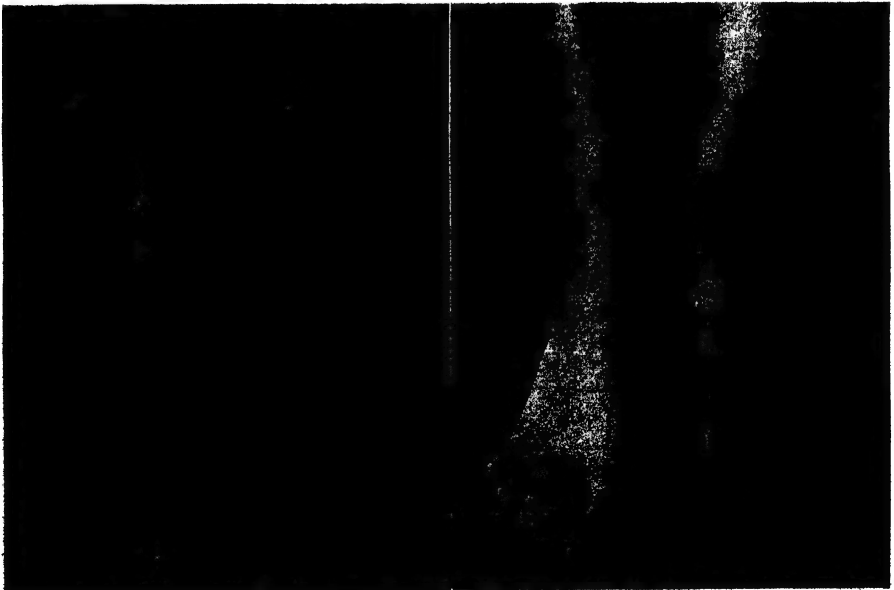
first the skin lesions are erythematous and somewhat resemble sunburn, but later they change to a reddish brown colour, a fine branny or sometimes coarse desquamation occurs about a fortnight later, and the underlying skin is thickened. Permanent pigmentation may develop in pellagrins who have been subject to repeated occurrences of dermatitis.

Stannus [221] suggests that the initial erythema in pellagra is due to changes in the posterior nerve roots resulting in the liberation of histamine or the "H" substance of Lewis at the periphery. This causes a local

PELLAGRA



FIGS. 84 and 85. Pellagra. A case admitted to the London Hospital under Dr. S. L. Simpson. The arm on the left shows the condition before treatment; brown pigmentation and thickening of the skin, which is cracked and glossy. The same arm, after a fortnight's treatment with a preparation containing the vitamin B complex, but not vitamin B₁, is shown on the right. The skin is now approximately normal.



FIGS. 86 and 87. Pellagra. The feet of the same patient shown in Figs. 84 and 85 before and after treatment with a vitamin B complex preparation. The skin in the photograph on the left is highly pigmented and shiny. The skin is

dilatation of vessels and an intercellular œdema, which prevents the escape of tyrosine which is converted into melanin.

The skin becomes scaly and over the legs and hands may sometimes present a typical appearance, which has been likened to cracked enamel or crazy paving (Figs. 83, 84). Sometimes the skin is uniformly smooth and shiny (Fig. 86). In severe cases the skin over a large area of the body may resemble that of a well-roasted turkey.

Hyperkeratosis (Figs. 94, 95) with callus formation is characteristic in chronic pellagrous dermatitis, and commonly appears over skeletal pressure points (knee, elbow, instep, front and back of the ankle), and may precede the exfoliating dermatitis and other manifestations of pellagra, particularly in those not exposed to sunlight. Indeed this may be noted by the potential pellagrin long before the prodromal appearances of the disease. This hyperkeratosis varies in appearance. Over the knees and instep the lesions may be wrinkled, or there may be fissures, or the hyperkeratosis may be nodular. The hyperkeratotic skin commonly shows pigmentation ranging from a light yellow, through brown to black.

These hyperkeratoses are an exaggerated response to irritation. They may also occur on the soles of the feet, although they do not necessarily occur over pressure points as they occur on bedridden patients. A fairly common type of hyperkeratosis is a diffuse thickening of the skin over the fingers, especially over the knuckles; it may be smooth and white or fissured, rough and pigmented.

Another skin manifestation is an ichthyosis-like change, which may be overlooked or attributed to chapping. It is worse in winter than in summer, but it may also be conditioned by exposure to heat. The usual site of these ichthyosis-like lesions is the antero-lateral aspect of the calves and less frequently the forearms. In a few cases the large thick plaques simulate alligator skin. A fine bran-like desquamation may also be seen.

The skin lesions sometimes become crusted and secondarily infected, particularly in natives, whose local remedies, *e.g.*, dung, usually make the condition worse.

The genital and anal regions are often affected and the lesions appear at the same time as those of the tongue and mouth. An irritating secretion is poured out by the vagina which may macerate the perianal region. The lesions are red, macerated and often infected, particularly with Vincent's organisms. These lesions occur in some fifty per cent. of females with severe pellagra [222].

In pellagra there is an over-activity of the sebaceous glands (dyssebacia), with the formation of inspissated sebum blocking the mouths of the sweat glands. This lesion, which according to Smith [222] occurs in twelve per cent. of pellagrins, is due to riboflavin deficiency (p. 333).

Lesions of Mouth and Lips. Pellagrous glossitis (Figs. 82, 96-98), which is common, is characterized by swelling and redness of the margins and tip of the tongue with indentations made by the teeth. Hyperæsthesia of the tongue frequently precedes objective signs. At times large red fungiform papillæ appear against a background devoid of filiform papillæ. As the disease progresses desquamation of the superficial epithelium leaves

a scarlet, smooth, dry and beefy-looking tongue (Fig. 82). The desquamation may be irregular giving the appearance of the "geographical tongue." During desquamation secondary infection with Vincent's organisms and monilia frequently occurs, producing a thick white coating, which is ultimately shed. As the tongue becomes red and swollen, fissures and

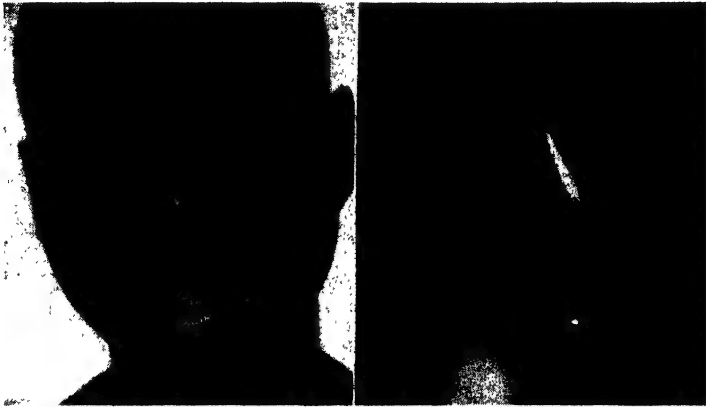


FIG. 88. Vitamin B Complex Deficiency before Treatment. Skin rough and dull, angular stomatitis, cheilosis, crusts on lips and eyelids, dry rugose desquamating scrotum.



FIG. 89. Vitamin B Complex Deficiency after Treatment. The same patient as in Fig. 88 after ten days' treatment with marmite, a yeast preparation containing the vitamin B complex.

aphthous ulcers develop on its surface—in fact aphthous ulcers should always suggest the possibility of a pellagrous aetiology. The inflammatory process extends to the buccal mucosa, gums, lips and pharynx, producing reddening and superficial ulcerations. In advanced pellagra biopsy of the tongue shows extensive fibrosis of the submucosa and the adjacent muscular tissue.

The lips are often red and scaly (cheilosis) and fissures appear at the corners of the mouth (angular stomatitis). These lesions are due to riboflavin deficiency and do not respond to nicotinic acid (see p. 329).

Mental Symptoms: In pellagrins mental symptoms develop in one-third to a quarter of the cases if untreated. It has been estimated that in Italy, when pellagra was rife, four to ten per cent. of pellagrins became

permanently insane. Symptoms are exceedingly varied. A feeling of tenseness, irritability, mental depression and emotional instability are fairly common. Patients weep without cause and insomnia is frequent. Melancholia, lethargy, and stupor are common, but confused states with hallucinations are also seen, as well as excitement, mania and delirium. The mental symptoms are particularly amenable to nicotinic acid therapy.

The mental symptoms of pellagra have been specially studied by Frostig and Spies [93], who describe the symptoms of the initial nervous syndrome. They are: hyperæsthesia to all forms of sensation; increased psychomotor drive; increased emotional drive with

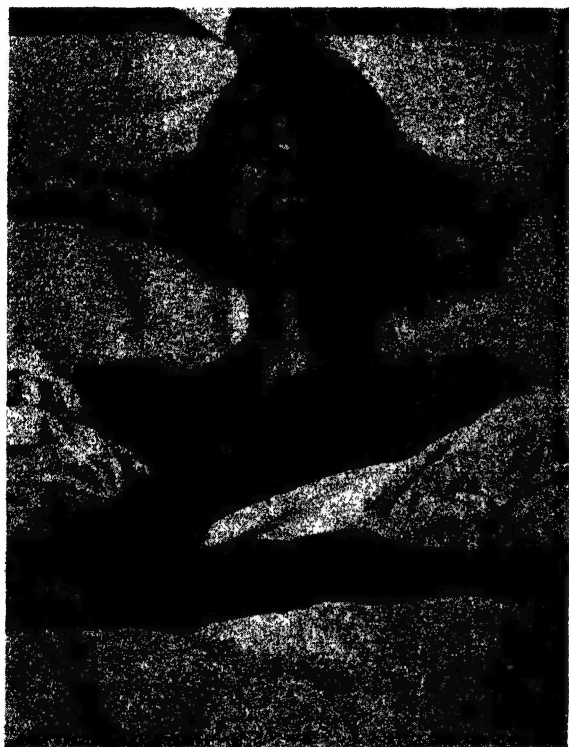


FIG. 90. Pellagra. A fatal case photographed in a London hospital. The dermatitis on the dorsal aspect of the hands is bilateral and sharply demarcated from the adjacent skin. There are pellagrous lesions on the forehead, cheeks and chin. The skin on the dorsum of the forearm is thickened and pigmented. The lesions of the lips and corners of the mouth resemble those of ariboflavinosis (p. 329).

a definite trend toward depression and apprehension; weariness and increased fatigue; headaches and sleeplessness; loss of memory; and confusion. In general the patients appear to have anxiety states with depressive features. There are also types in which excitement, mania, and delirium may occur.

Psychosensory disturbances occur in all the special senses. Patients dislike bright light and colours, noises cannot be tolerated, music upsets them, odours and tastes may be so disagreeable as to cause nausea and

vomiting. The patients can be described as being "on edge," irritable and tense. Many abnormal skin sensations are observed. Prominent complaints are dizziness, difficulty in maintaining balance, flickering stars and dark spots in front of the eyes.

The psychomotor drive is increased—the patient is fidgety, moves about a great deal, and is quarrelsome. He complains that a sudden noise or flash of light makes him jump and twitch. Emotional reactions are increased. The patient is more excitable and sensitive than usual; he is often depressed, sad and gloomy, and he is in a constant state of apprehension. Many patients express various fears, frights and phobias, although they may try to suppress them. The emotional outlook is gloomy and pessimistic and imminent danger is constantly expected.

In spite of the increased motor drive and restlessness the patients complain of weakness and fatigue. They tire readily at their work. There is a conflict between restlessness and fatigue, with the former often prevailing. Sleeplessness is



FIG. 91. Pellagra. A pellagrin showing mental symptoms. Note the characteristic dull, lifeless vacant stare and the dilated pupils. There are pellagrous lesions on the face and forearm. Melancholia, lethargy and stupor are common.

also a common symptom, the patient falling asleep between 12 p.m. and 2 a.m. and waking again at 5 a.m. Sick headaches are common, resembling those of migraine, and occurring suddenly. The pain is localized in the forehead and temples and is accompanied by scintillating scotomata. As in migraine nausea and vomiting are frequent. Developing pellagra often causes a breakdown in personality. Individuals previously strong, courageous and enduring become shaky, weary and apprehensive before clinical pellagra can be diagnosed. Severe pellagrous psychoses occur in ten per cent. of untreated pellagrins. The patient may have periods of depression and apprehension followed by confusion, hallucinations,

delirium, disorientation, and confabulation. A paranoid condition is often observed. Tremor, jerky movements and rigidity of the body may accompany these symptoms. In cases with severe depression the patient may have a mask-like expression and sit in one position staring into space for hours without moving.

The mental symptoms may precede the other symptoms of pellagra, so that a potential pellagrin may easily be diagnosed as "neurasthenic" or a paranoid. This is important because the mental condition clears rapidly in a few days with nicotinic acid therapy, whereas a case of true neurasthenia or paranoia remains unaffected.

Neurological Lesions. Deprivation of nicotinic acid is not responsible for all the symptoms of pellagra. In most pellagrins the alimentary tract and skin lesions are associated with neurological ones, manifested by pain in the calves, numbness, burning or itching of the extremities, weakness, difficulty in walking, dizziness, vertigo, lassitude, fatigue and occasionally tinnitus. Absent knee jerks, numbness, formication and retrobulbar neuritis have been reported.

Stannus [221] states that burning hands and feet are most constant symptoms in pellagra and almost pathognomonic. The neurological manifestations are not relieved by nicotinic acid, but are by preparations rich in the vitamin B complex. Kark [226] states that the burning feet are made worse by treatment with nicotinic acid alone. Spies and his co-workers [95] believe that the neurological lesions are a manifestation of vitamin B₁ deficiency and claim that considerable improvement in these lesions occurs if the patients are given vitamin B₁ or cocarboxylase. Stannus [221] thinks that the burning pains in the extremities are due to riboflavin deficiency and that the retrobulbar neuritis may be due to nicotinic acid and riboflavin deficiency. Fitzgerald Moore [223], Landor and Pallister [224] and Wilkinson and King [225] describe nutritional retrobulbar neuritis (the latter prefer to call it amblyopia) occurring in pellagrous subjects in Africa, the Malay States and Ceylon. The condition cleared up with yeast extracts, autoclaved yeast and liver. Wilkinson and King's cases responded more rapidly and specifically to nicotinic acid than to yeast.

Other Manifestations. Anæmia. Anæmia is common among pellagrins, although its occurrence is inconstant and it bears no direct relationship to the achlorhydria. The type varies; hypo- and hyper-chromic varieties and various colour indices have been recorded.

Genitourinary. Burning on urination, albuminuria [220], casts and indicanuria may be present. Libido is decreased, sterility common, and menstruation scanty. Porphyrinuria occurs, but is an inconstant finding and of no diagnostic importance (p. 384).

Cardiovascular. The blood pressure is normal or slightly subnormal, and in severe cases the pulse rate is increased. Death in severe cases frequently occurs after vasomotor collapse and syncope.

Mainzer and Krause [87] studied the electrocardiographic records of a number of pellagrins, and in about three-fifths the electrocardiogram was abnormal. That these abnormalities had a causal relationship to pellagra is demonstrated by the fact that their development ran parallel to the

clinical course of the disease, and by the rapid disappearance in some cases after nicotinic acid treatment. Tachycardia was observed mostly when the disease was at its height, and bradycardia during convalescence. The most frequent electrocardiographic changes were a low voltage and notching of the ventricular complex, inversion of the T wave and shorten-



FIG. 92. Pellagra in a Child. Note the butterfly-like distribution of the dermatitis on the face. Pigmentation is present on the exposed parts and is absent from those covered by clothing.

ing of the P-R interval. These changes are, however, not characteristic of pellagra.

Rachmilewitz and Braun [218] also noted marked changes in the electrocardiogram, particularly in the T waves, which were flat in T_1 and inverted in T_2 , T_3 , T_4 , and were reversed by administering nicotinic acid. They suggest that nicotinic acid deficiency has a specific effect on the heart muscle, possibly by interfering with the coenzyme system.

Pellagra sine Pellagra. Forms of pellagra have been described in which skin lesions are absent—*pellagra sine pellagra*. The outstanding lesions

are stomatitis, glossitis, cracked lips and sores in the corners of the mouth, and although it is claimed that some cases have responded to treatment with nicotinic acid [89-92], it seems more likely from recent work that these symptoms are due to riboflavin deficiency (p. 329).

Infantile Pellagra. It has been thought by some that true pellagra is rare in children. There are many references to "infantile pellagra," suggesting that this disease is not the same as that met with in the adult [95]. Indeed many writers have given names such as Gillan's œdema, Williams' disease, "pellagroid beriberi," nutritional œdema, and even "infantile scurvy" to conditions, which on close study appear to be none other than infantile pellagra, *i.e.*, pellagra occurring in infants and young children.

The following table modified from Kark [226] shows the comparative incidence of signs in adult and infant cases of pellagra. The differences are mainly of degree, the infant cases being more acute than the adult. Infantile pellagra is also characterized by a marked œdema.

Signs	Adult.	Infant.
<i>Dermatitis</i>		
"Classical"	+++	+++
Phrynoderma	++	—
Seborrhœic	+	—
<i>Mouth</i>		
Gingivitis	+	+
Glossitis	+++	+++
Stomatitis	+++	+++
Cheilosis	+++	+++
<i>Eyes</i>		
Blepharitis	++	+++
Conjunctivitis	+	+++
Keratitis	+	—
<i>Lesions of perineum, scrotum, vulva</i>	++	+++
<i>Gastro-intestinal</i>		
Diarrhœa	+++	+++
Constipation	+	—
<i>Nervous system</i>	++	++
<i>Mental changes</i>	++	+++ (irritability)
<i>œdema</i>	+	+++

Outstanding.
 ++ = Common, but not dominant.
 + = Sometimes present.

The principal manifestations of pellagra in the native infant and young child are œdema, diarrhœa, photophobia, hypotonia, irritability and a dermatitis that differs from the adult type. œdema is characteristic in the infantile type, but is not commonly present in the adult. The mental change in the adult is mainly one of depression, with the characteristic sad

faces ; the child is usually vexatious and irritable. In the adult the main neurological manifestation is a burning sensation in the feet and hands ; in the child the extremities are tender. In the infant and child there is a sloughing type of dermatitis, commonly noted in the body creases, face, hands, elbows, buttocks and abdomen. It is not uncommon to find extensive raw areas in the perineum, groins, buttocks and between the lower jaw and neck. In natives there is a general pallor of the body, most marked on the face, with which is associated a patchy distribution of black hyperpigmented and pale hypopigmented areas. Angular stomatitis and inflammation of the buccal mucous membrane are common in both infants and adults. Swollen red-dened gums occur in children and adults. In the infant eye signs such as blepharitis and conjunctivitis are more common than in the adult. Diarrhoea with mucus in the stools is an almost constant finding in the infant ; in the adult some have constipation and others diarrhoea.

Spies and his co-workers [51, 86] have described the manifestations of pellagra in children in pellagrous families in the United States. These differ somewhat from those previously described in native children. The following history is typical: below normal

weight and height for years ; slow progress at school ; lack of interest and ability to concentrate ; complaints of poor appetite, vomiting, indigestion, constipation, sore tongue and lips ; the child is cross, fretful, and cries easily. Careful inquiry often reveals that the mother was inadequately nourished during pregnancy and while nursing the child. The children also show a preference for foods rich in carbohydrate and refuse others even when available. These children rapidly improve on a full diet with nicotinic acid and supplements of other vitamins.

Diagnosis. The diagnosis of full-blown pellagra is made on the

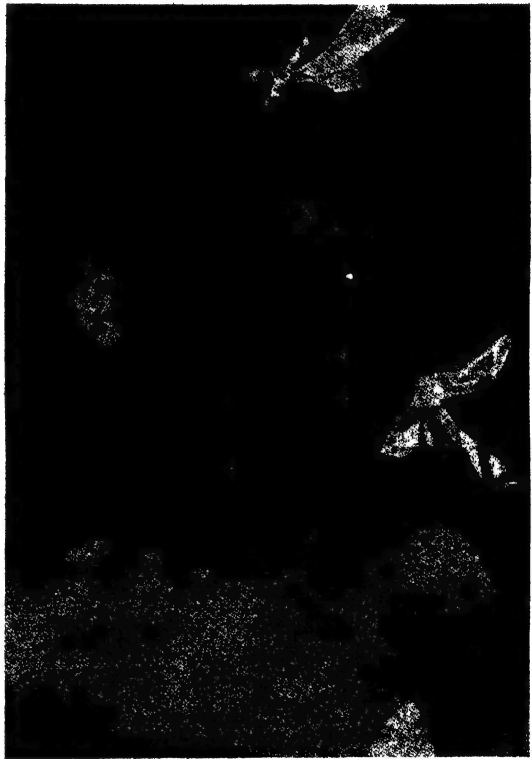


FIG. 93. Pellagra in an English Schoolgirl. The dermatitis is present on the forehead, nose, cheeks, chin, neck and hands. There are lesions at the corners of the mouth as in ariboflavinosis (p. 329).

dermatitis, stomatitis, glossitis, mental and gastro-intestinal symptoms, the dietary history and response to the therapeutic test with nicotinic acid and foods rich in the vitamin B complex. The diagnosis is not difficult in cases with a characteristic pellagrous dermatitis, especially if this is symmetrical and shows a seasonal exacerbation, but in the absence of the latter, predominating gastro-intestinal symptoms, glossitis and stomatitis may simulate sprue (p. 354). The neuropathy, glossitis and macrocytic anæmia of pellagra without dermatitis may cause confusion with pernicious anæmia in the tropics and with subacute combined degeneration. The anæmia in pellagra is of the normocytic hypochromic type and the hæmoglobin commonly fifty to seventy per cent. Achlorhydria is common. A history of repeated attacks of the disease, particularly in the spring, and of dietary deficiency helps in the diagnosis. The skin lesions may be mistaken for those of acrodynia, erythema multiforme, erythema solare, occupational dermatitis, syphilis, lupus erythematosus and toxic dermatitis. The nervous manifestations have to be differentiated from those of hysteria, ergotism, lathyrism and general paralysis of the insane. In old people with arteriosclerotic changes and accompanying mental symptoms there may be lesions of the hands and face, which may cause confusion in diagnosis. Other conditions in which some of the signs and symptoms of pellagra may appear are tuberculous enteritis, chronic pancreatitis, stomatitis of varying ætiology, neurasthenia and Vincent's angina.

The Plummer-Vinson syndrome has some symptoms in common with pellagra—glossitis, dysphagia and anæmia. The possibility should always be borne in mind that pellagra may be associated with other diseases such as syphilis, tuberculosis, tropical diseases and conditions mentioned on p. 395, which may act as predisposing causes, and to which pellagra may be secondary.

Laboratory Tests for Diagnosis of Nicotinic Acid Deficiency. Blood and urine estimations of nicotinic acid and codehydrogenases I and II have proved disappointing as a means of detecting nicotinic acid deficiency (p. 389). The normal variations in health are not sufficiently well defined for standards to be laid down. Whilst some have stated that a low excretion of nicotinic acid, *e.g.*, 3 mg. daily, occurs in pellagrins [11, 144], others are of the opinion that the estimation of blood and urinary nicotinic acid is useless in the diagnosis of pellagra and disturbed nicotinic acid metabolism [50, 84, 227].

Provided that coffee, tobacco, nuts and legumes are excluded, it is stated that the urinary excretion of trigonelline is a measure of nicotinic acid intake [147, 228], although this is challenged by Ellinger and Coulson (p. 389) [282, 283]. Most observers state that in deficient subjects a marked decrease in the urinary excretion of trigonelline occurs. Perlzweig and his co-workers [148] and Goldsmith [228] suggest the use of a test dose method for evaluating the nutritional status of nicotinic acid. Perlzweig [148] gives a test dose of 500 mg. of nicotinamide orally and examines the urinary excretion of nicotinic acid and trigonelline for the next twelve hours. In normal subjects the excretion rises from about 10 to 100 mg. after the test dose of nicotinamide. In patients suffering from nutritional deficiency the excretion after a test dose falls to values from 40 to 71 mg.

Goldsmith [228, 281] determines the excretion of trigonelline in a six-hour period after the administration of an oral test dose of 300 mg. of nicotinamide, which she states is excreted almost entirely as trigonelline. The normal figure ranges from 20 to 45 mg., with an average of 30 mg.; in pellagra the figures are 3 to 35 mg., with an average of 16 mg.

Ellinger and Coulson [282, 283] have shown that nicotinic acid and its derivatives are partly excreted as nicotinamide methochloride (p. 389), but so many factors effect the excretion of the latter that it is doubtful if its measurement in the urine is of value for determining the nutritional status of the individual with respect to nicotinic acid.

In 1937 Beekh, Ellinger and Spies [25] employed the Ellinger-Dojmi colour reaction for the estimation of porphyrin as an aid in the diagnosis of pellagra, in the belief that in the latter there was an increased porphyrin excretion. The test is performed as follows: to 10 c.c. of urine, about 0.2 c.c. of glacial acetic acid is added to make the pH 4. Then 15 to 20 c.c. of ether are added to the mixture in a separating funnel and the ether layer separated after shaking. The ether layer is washed with 10 c.c. of distilled water and 3 c.c. of twenty-five per cent. hydrochloric acid added. A pink to purple colour develops and may be estimated colorimetrically. Originally thought to be specific for porphyrin, this colour reaction is due to urorosein [177], the presence of which in urine is not pathological. It may occur in patients with irradiation sickness, arthritis, and gastrointestinal disorders. Watson and his co-workers [177] could not correlate the pink colour of the Beekh, Ellinger, Spies reaction with other evidences of nicotinic acid deficiency.

Najjar and Holt [55] claim to have devised a specific chemical reaction whereby nicotinic acid deficiency can be quantitatively measured. They state that when normal urine is adsorbed on zeolite with potassium chloride, the eluate on treatment with caustic soda and butanol develops a bluish fluorescence, which can be estimated by means of a fluorophotometer. The materials causing this fluorescence, referred to as F_1 and F_2 , are often absent from the urine of pellagrins. In addition the urine of the latter gives a whitish-blue fluorescence without the addition of alkali. Sargent, Robinson and Johnson [286], however, state that the presence or absence of F_1 in urine bears little or no relation to the nutritional state, and that it fluctuates with the intake of vitamin B_1 . In most subjects there is an increased excretion of F_2 when nicotinic acid and other related compounds effective in pellagra (p. 383) are administered [145], and an increased excretion of F_1 after the ingestion of vitamin B_6 [146]. Huff and Perlzweig [229, 273] believe that the cation of N-methyl nicotinamide is the precursor of F_2 , the methylation probably occurring in the liver [230]. Najjar and White [274, 275], however, who have isolated F_2 in crystalline form state that it is derived from N-methyl nicotinamide α -carbinol and is probably the butyl ether of this compound. Coulson and Ellinger [283] state that it is probably a mixture of *p*- and *o*-carbinol of N-methyl dihydronicotinamide. N-methyl nicotinamide has a preventive and curative action in black tongue [276].

Najjar [277] has devised a test for nicotinic acid deficiency based on the measurement of N-methyl nicotinamide chloride in the urine. As

N-methyl nicotinamide is conveniently prepared it has been used as the standard of reference, the urinary output of N-methyl derivative being expressed in terms of micrograms of N-methyl nicotinamide chloride, rather than in arbitrary fluorescent units of F_2 . An oral test dose of 100 mg. of nicotinic acid is given and the urinary excretion of N-methyl nicotinamide chloride during the following four hours estimated. According to Najjar, normal individuals excrete 2,100 to 4,000 micrograms of N-methyl nicotinamide four hours after the test dose, while an excretion of less than 2,100 micrograms is probably indicative of nicotinic acid deficiency.

Coulson, Ellinger and Smart [288] have used the excretion of nicotinamide methochloride in the urine seven hours after the ingestion of a test dose of 100 mg. of nicotinamide as an index of nicotinic acid nutrition in R.A.F. personnel. They state that results obtained with this test are related to standards of nutrition. Subjects receiving the best food excreted the greatest quantity of nicotinamide methochloride after a test dose of 100 mg. of nicotinamide. The nicotinamide methochloride of Coulson and Ellinger appears to be the same as the N-methyl nicotinamide of Najjar.

The estimation of F_2 or nicotinamide methochloride in the urine as an index of nicotinic acid nutrition requires further investigation before it can be accepted without question. Sargent, Robinson and Johnson [286] agree that the level of F_2 in the urine is correlated fairly well in most cases with the level of nicotinic acid nutrition, although they state that some subjects on a diet adequate in nicotinic acid may excrete no F_2 and that fasting for sixteen days may raise the F_2 excretion to high levels. A small number of subjects examined by them failed to show an increased excretion of F_2 after a test dose of nicotinic acid. Mickelsen [289, 300] studied two groups of young men on daily intakes of 10 mg. and 20 mg. of nicotinic acid respectively. Over a period of nearly six months he failed to observe any significant difference in the excretion of F_2 in the two groups. Furthermore there was no difference in the F_2 excretion after test doses of 10 mg. or after cutting down the nicotinic acid intake to 0.25 mg. daily. It is clear from these observations that tests involving the estimation of F_2 or nicotinamide methochloride in urine must be interpreted with considerable caution, particularly in the absence of clinical manifestations of nicotinic acid deficiency.

Pathology of Pellagra. Post-mortem the only external appearances that are of diagnostic value are the skin and mouth lesions; emaciation occurs late. The pathological lesions are frequently obscured by complicating diseases, such as bacillary dysentery and tuberculosis.

The most striking histological skin changes are hyperkeratosis (Figs. 94, 95), parakeratosis, acanthosis, hyperplasia of the sweat glands, dilatation of the papillary blood vessels, moderate lymphatic infiltration, and plugging of the hair follicles with dry sebaceous material [232, 233]. Slight oedema of the deeper portions of the epidermis occurs and cells of the basal layer undergo multiplication. The skin lesions are sharply limited to the zone between the corium and epidermis. Generally speaking the microscopic picture is similar to that found in chronic inflammatory diseases of the skin. The skin changes are to a considerable extent rever-

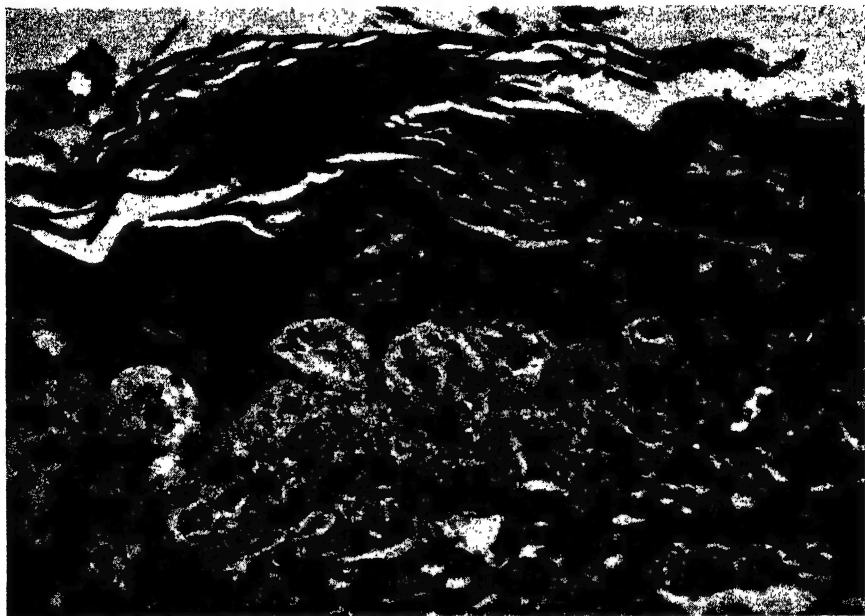


FIG. 94. Skin in Pellagra. Low-power photomicrograph showing hyperkeratosis, oedema and distortion of the rete pegs of the epidermis.



FIG. 95. Skin in Pellagra. Low-power photomicrograph showing hyperkeratosis, atrophy of the epidermis, and oedema of the cutis.

sible and may represent a specific response on the part of the skin to a deficiency of nicotinic acid and possibly other members of the vitamin B complex.

Degeneration of the nerve fibres of the skin in early pellagra has been described. Vesicular formations in the epidermis may occur, and if they become infected the epidermis sloughs off, leaving an atrophic or horny lamellar corium. The pigmentation is due either to the formation of melanin in the malpighian and basal layers of the epidermis, or to the formation of granules of an iron pigment in the epidermis. In old lesions atrophy and reduction in the cells of the malpighian layer occurs.

As a rule little can be seen macroscopically in the gastro-intestinal tract. Greenfield and Holmes [71] describe the *état mamelonné* of chronic gastritis; enteritis has been described. The walls of the colon may be thickened and red, and covered with pseudo-membranous patches and minute grey cysts, formed by distention of the crypts of Lieberkühn.

Vedder [231] states that the gastro-intestinal tract is considerably inflamed and frequently ulcerated, particularly in the small intestine, colon and rectum. To what extent these changes are terminal ones following emaciation is difficult to decide. Fatty degeneration of the viscera and atrophy of the thyroid and adrenal glands have also been described.

The nervous lesions are late in appearance. The most common lesions in the spinal cord consist of areas of scattered symmetrical myelin degeneration in various tracts, particularly in the posterior and lateral columns. The peripheral portions of the fibres are frequently spared. In most cases the afferent tracts are more affected than the efferent, although in occasional cases no lesions in the posterior columns have been observed. Chromatolysis of the posterior root ganglia with loss of Nissl granules is most frequent in the dorsal, lumbar and lower cervical regions. In the grey matter there is pigmentation of the cells of the anterior and posterior horns, the latter appearing to be degenerated from the cervical region downwards. The cells in Clark's column are particularly affected. In the anterior horns in the lumbar region the cell bodies are swollen and the nucleus is displaced to the periphery as a result of chromatolysis [231]. In the brain the frontal lobe is most frequently involved. The large pyramidal cells, in scattered foci, show chromatolysis with nuclear displacement and accumulations of fat. In late cases gliosis occurs. There is some wasting of the brain with excess fluid in the ventricles. Pathologically the lesions bear some resemblance to those of subacute combined degeneration, which is related to pernicious anæmia. The possible connection between pernicious anæmia, pellagra and sprue has been mentioned previously (p. 352).

Prognosis and Treatment. Most cases develop in late winter or spring, become more and more severe for two or three months and then slowly improve. The patient may recover completely or vague symptoms may remain. Recurrences may occur every spring, and with each attack the patient becomes weaker and more emaciated until death occurs, in the average untreated case, in about five years. Acute cases have been described, particularly in children, in which death may occur in

the first attack owing to severe gastric and nervous involvement. Recovery readily occurs following effective treatment, but relapse is common.

Nicotinic acid, sodium nicotinate, or nicotinamide relieve the acute mental symptoms in a dramatic fashion, and also improve the alimentary tract and skin lesions, but have little effect on the neuropathy, or on the lesions of the lips and face, which respond to vitamin B₁, riboflavin and the vitamin B complex. Nicotinamide has an advantage over nicotinic acid in that it does not produce vasodilatation of the skin, flushing, itching and other reactions (p. 387); the dosage of nicotinamide is the same as that of nicotinic acid. Reactions from the latter are less likely if it is given after food.

Treatment of Mild Cases. Patients with mild or subclinical pellagra recover rapidly on a diet containing adequate quantities of nicotinic acid and other members of the vitamin B complex. Plenty of red meat, meat extracts, liver, eggs, fresh vegetables, milk and yeast extract or brewers' yeast should be incorporated in the diet. Some commercial yeasts may not supply sufficient nicotinic acid to prevent pellagra [278]. Small doses of nicotinic acid, 25 to 50 mg., two or three times daily after meals are helpful. Exposure to direct sunlight, rough clothing, and skin trauma should be avoided. If there is any evidence of vitamin B₁ deficiency, *i.g.*, anorexia or polyneuropathy, 8 to 10 mg. of vitamin B₁ should be given daily.

Treatment of Moderately Severe Cases. A case of moderately severe pellagra should be confined to bed until the skin lesions have disappeared. The same general and dietary treatment as described in the mild cases should be followed. Nicotinic acid or its amide is given in doses of 100 mg. three times a day after food. The lesser-known members of the vitamin B complex are best administered in the form of yeast (1 oz. to 6 ozs. daily), or yeast or liver concentrates. Crude liver concentrates are given in doses of a tablespoonful three times daily. Supplements of vitamins A and D (fish liver oils), C and iron (ferrous sulphate gr. 3 t.d.s.) should be included and a high calorie diet (2,500–4,000 calories) provided. Riboflavin, 3 to 10 mg. daily, and vitamin B₁, 5 to 10 mg. twice daily, after meals, help to control associated riboflavin and vitamin B₁ deficiencies. After recovery the dosage of nicotinic acid is reduced to a maintenance dose of 50 mg. once or twice daily.

Treatment of Severe Cases. Severely ill patients should be hospitalized and treated as emergencies, as they may collapse and die within a day or so. Diarrhoea and dementia are often present so that normal feeding has to be abandoned at first. The patients are usually dehydrated from the diarrhoea and the associated glossitis, dysphagia, anorexia and vomiting prevent the ingestion of food. The administration of vitamin concentrates by mouth is therefore useless in the early treatment of the disease. The patient is given intravenous infusions of five per cent. glucose in normal saline in doses of 500 to 1,000 c.c. two or three times daily for the first day or so. This may be continued if the patient cannot take fluids by mouth or has severe diarrhoea. It is not advisable to give yeast at this stage as it cannot be retained and makes the diarrhoea worse. Nicotinic

acid or the amide is given in the saline drip in doses of 10 to 20 mg. as a single dose, this amount being repeated at hourly intervals. Too large a quantity at once may cause a reaction. Some workers have found six intravenous injections of 50 mg. a day (total 300 mg. daily) satisfactory. If nicotinic acid is tolerated by mouth and there is no severe diarrhoea, 300 to 500 mg. daily for a week is given orally in divided doses. Earlier workers gave 1,000 to 1,800 mg. of nicotinic acid daily by mouth in severe cases. To the saline glucose drip may be added 100 mg. of vitamin B₁, 50 mg. of riboflavin and 200 mg. of vitamin C. Intravenous therapy is continued until the diarrhoea and vomiting have subsided. The patient is then given a liquid diet supplemented by nicotinic acid (300 to 500 mg. daily), yeast, meat extracts, liver extract and vitamin B₁ and riboflavin in the doses mentioned. Occasionally patients do not respond readily to nicotinic acid; in such cases favourable results are often obtained by crude liver extracts given parenterally in doses of 5 c.c. daily. Liver extracts and stomach extract are sometimes life-saving in severe cases [270]. When improvement has occurred a maintenance dose of 100 mg. of nicotinic acid once or twice daily usually suffices. Within one to three days of beginning treatment the fiery tongue and soreness of the anus and vagina subside; the dermal erythema blanches; the acute mental manifestations often vanish overnight; and the papillae of the tongue regenerate after seven to fourteen days. The diarrhoea, however, may persist for five to ten days. The mental changes unless of long standing are usually reversible.

Symptomatic Treatment. A mild alkaline mouth wash may be used for the stomatitis, but the teeth should not be brushed as the gums are tender. For the dermatitis dressings of calamine may be used; if there is secondary infection this may require local treatment. Sedatives may be required at first for the uncontrollable mental patients, who are, however, usually amenable after the first few days on nicotinic acid therapy. Tinct. opii 30 minims every four hours helps to control the diarrhoea in the early stages. If the hæmoglobin is below fifty per cent., blood transfusions may be given, and iron as ferrous sulphate 3 mg. t.d.s. administered to control the anaemia. It is important to correct achlorhydria if present, as this interferes with the absorption of iron and vitamins.

Relapses are common once the patient passes out of the care of the hospital or physician. This is not surprising. How can the pellagrin, whose disease is usually due to poverty, afford a diet containing adequate protective foodstuffs? The problem of pellagra is not medical but economic. In America pellagrins are encouraged to keep chickens or a cow or to cultivate kitchen gardens or small holdings. Spies [88] states that a mixture of twenty-five per cent. brewers' dried yeast, sixty-seven per cent. peanut butter and eight per cent. peanut oil in daily doses of 2 ounces is an inexpensive and palatable dietary supplement and tends to prevent pellagra, beriberi and riboflavin deficiency.

Nicotinic Acid Psychoses. It is believed by Jolliffe and others [99, 100] that nicotinic acid deficiency is responsible for an encephalopathic syndrome, which has a mortality of eighty-nine to one hundred per cent. unless treated. A hundred and fifty cases were studied by Jolliffe, who observed that the condition was associated with deficiency diseases,

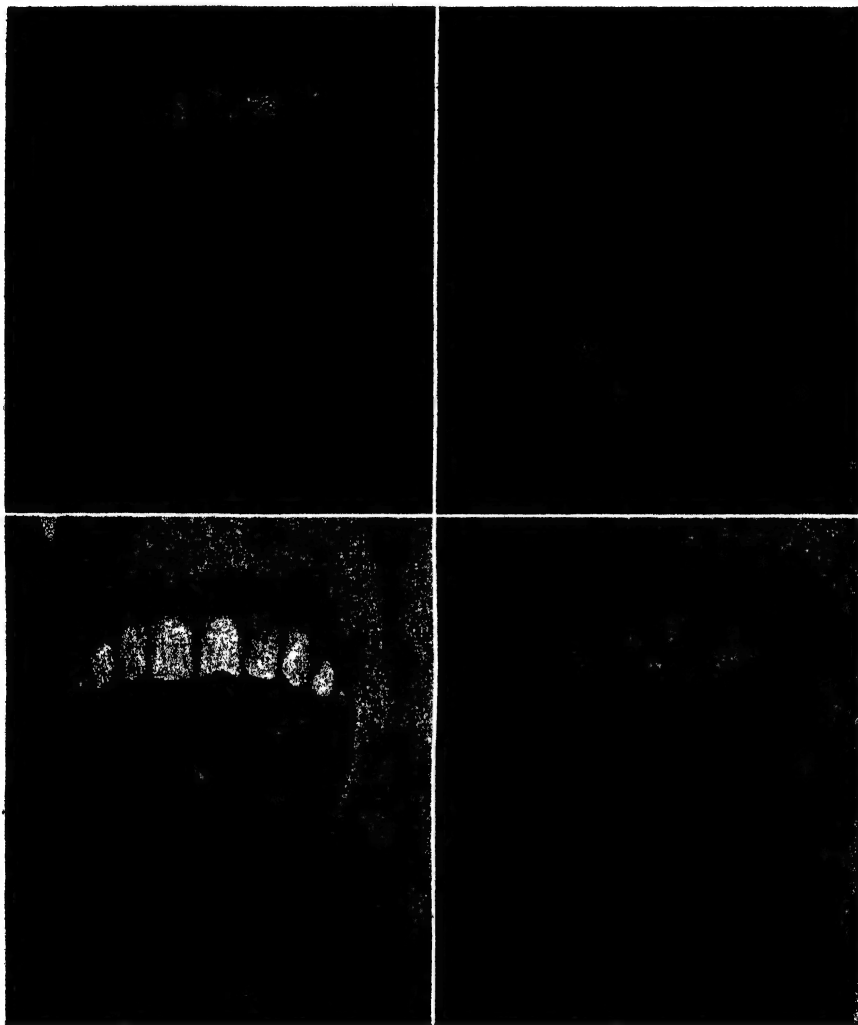
particularly with deficiency of the vitamin B complex. The clinical picture is characterized by clouding of consciousness, changing cogwheel rigidities, and uncontrollable sucking and grasping reflexes; there may also be oculomotor disturbances varying from bilateral nystagmus to complete ophthalmoplegia. Sydenstricker [101] adds hebétude grading into profound stupor, delirium, and agitated depression. According to Jolliffe this syndrome may occur independently or in association with pellagra or with polyneuritis or with both. Sydenstricker, however, observed it in nineteen cases in the absence of a complete syndrome of pellagra or a history of pellagra.

This syndrome treated by hydration and vitamin B₁ had a mortality of one hundred per cent. in one group, and a mortality of sixty-two per cent. when treated with hydration and the entire B complex. In the hands of Jolliffe this was reduced to 81.8 per cent. with nicotinic acid, which was injected in 100 mg. doses up to a total of 500 mg. a day; later, this was increased to 1,000 mg. a day in 100 mg. doses. Sydenstricker used 100–300 mg. of sodium nicotinate in normal saline containing five per cent. glucose, which was given intravenously, and 100 mg. of sodium nicotinate intramuscularly. The cure was described as dramatic. Recovery occurred within a few days when the patients were given a high calorie diet supplemented by nicotinic acid and the vitamin B complex, vitamin B₁ and riboflavin. A control group presenting stupor of demonstrable origin was employed by Sydenstricker; they did not respond. The workers on this subject are convinced that a therapeutic test with nicotinic acid is justifiable in cases of unexplained hebétude or unconsciousness with a bad dietary history.

Jolliffe believes that this encephalopathic syndrome results from a complete and acute nicotinic acid deficiency, while the pellagra syndrome represents a partial and more prolonged deficiency of nicotinic acid. Patients showing the encephalopathic syndrome but no signs of pellagra represent a complete nicotinic acid deficiency which develops so rapidly that the changes of pellagra do not have time to occur.

Sydenstricker [284] also draws attention to the psychoses formerly classified as "toxic," "exhaustion delirium" and "psychosis, cause undetermined" not infrequently seen in general hospitals and seen sometimes after surgical operations or after delivery. In most cases there is no history of frank dietary deficiency, although some patients are alcoholic, some have been dieted for medical or surgical reasons; others have their vitamin requirements increased by fever or infection. Intravenous saline and glucose infusions without food by mouth sometimes precipitate an attack. The onset of delirium, hallucinations or mania is abrupt, or after a short period of confusion. An important diagnostic sign is the fluctuation of the condition, the patient improving or relapsing for no obvious reason. The tongue is frequently dry, clean and red—the so-called "toxic tongue." Rarely are there any classical signs of nicotinic acid deficiency present. Patients, particularly middle-aged or elderly, showing mental confusion, delusions, hallucinations, stupor, manic excitement and confabulation are often admitted to hospital with a provisional diagnosis

THE TONGUE IN NICOTINIC ACID AND RIBOFLAVIN DEFICIENCY



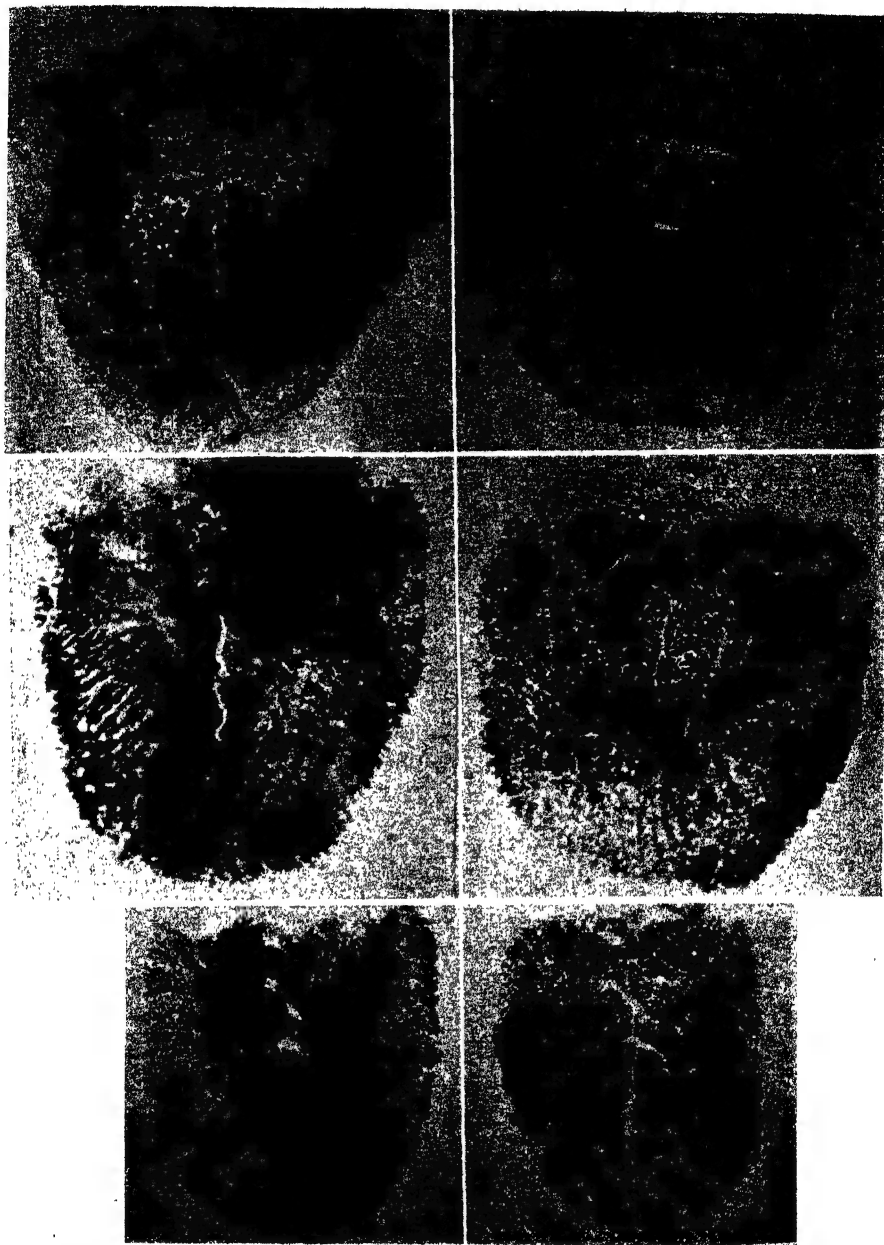
FIGS. 96 to 99. The Tongue in Nicotinic Acid and Riboflavin Deficiency.

FIG. 96 (*upper left*). Hypertrophy of the papillæ in a patient with nicotinic acid deficiency.

FIG. 97 (*upper right*). Tongue, which was fiery red, showing atrophy of the papillæ. From a case of nicotinic acid deficiency.

FIG. 98 (*lower left*). Bald atrophic tongue due to nicotinic acid deficiency.

FIG. 99 (*lower right*). Tongue showing fissuring and hypertrophy of some of the papillæ from a case of riboflavin deficiency. The tongue was magenta coloured.



FIGS. 100 TO 105. Tongue Prints from Cases of Nicotinic Acid Deficiency.

FIGS. 100 AND 101 (upper left and right). Tongue print showing progressive atrophy of the papillae.

FIGS. 102 AND 103. (middle left and right) Tongue print showing atrophy of the papillae on the left, with improvement on the right two weeks after treatment with nicotinic acid.

FIGS. 104 AND 105 (lower left and right). Tongue print of same case showing return of more papillae with vitamin therapy.

of uræmia, arteriosclerotic or senile dementia, neurosyphilis, drug intoxication, or a cerebral vascular accident. Some are even admitted to mental wards. Gottlieb [285] has described several patients admitted to a London hospital under such circumstances. Wexbeg [98] points out that some cases of senile dementia have nutritional deficiency as a background. These respond to treatment with nicotinic acid, which if given in sufficiently large doses produces a dramatic improvement in the mental condition in twenty-four to forty-eight hours. For immediate treatment Sydenstricker [284] suggests 100 mg. of nicotinic acid or 80 mg. of nicotinic acid amide every hour for ten hours during the first two days, continuing this dosage longer if necessary. This is given by mouth, stomach tube, or parenterally if the patient is stuporous or unco-operative. Once improvement sets in the daily dosage is reduced to 100 mg. of nicotinic acid five times a day, or 150 mg. of nicotinamide daily. Later, 25 mg. of nicotinic acid three times daily should be sufficient. Sydenstricker also gives vitamin B₁ in doses one-tenth of that of the nicotinic acid, *i.e.*, 50 mg. daily at first. Yeast in quantities of 15 to 80 gm. daily or other sources of the vitamin B complex such as yeast extract or wheat germ preparations are added to the diet.

Mainzer and Krause [121] gave large doses of vitamin B₁ in a case of delirium tremens associated with severe gastro-intestinal symptoms without effect, but the administration of nicotinic acid in doses of 0.5 gram daily made all pathological manifestations disappear within twelve hours. They believe that the prompt response to nicotinic acid favours the assumption that lack of vitamin is an important factor in the development of delirium tremens. May [122] also observed that nicotinic acid in daily doses of 600 mg. brought about improvement in four cases of severe psychosis with pellagrous dermatitis accompanying chronic alcoholism.

Sydenstricker and Cleckley [158] report that thirty-eight patients in stuporous states or in active psychoses without evident cause showed prompt and very often impressive improvement after treatment with nicotinic acid, in total dosage varying from 100 mg. to 4,500 mg. orally. Pellagra and other deficiency states were absent. Sydenstricker and Cleckley believe that the symptoms of many cases of toxic psychosis, exhaustion delirium and unexplained clouding of consciousness, may be relieved by nicotinic acid. In some cases very large amounts of nicotinic acid, *e.g.*, 4,500 mg., were necessary to obtain satisfactory results.

Lingual Manifestations of Nicotinic Acid Deficiency. Kruse [96] claims that the examination of the tongue affords a simple, convenient and objective method of detecting nicotinic acid deficiency. Combining naked-eye examination with biomicroscopy he states that all forms, degrees and stages of the condition can be noted and graded. Kruse divides the tongue lesions into acute and chronic, which may occur together, that is an acute deficiency may be superimposed on a chronic one. In the acute form vascular hyperæmia and proliferation, hypertrophy and extinction occur successively in the tongue papillæ, first in the fungiform and later in the filiform. The vascularity and hypertrophy of the fungiform papillæ impart to the tongue a stippled and then strawberry appearance, depending on the degree and stage of deficiency. Then redness, swelling, marginal

indentation and baldness appear. If the condition is intense the tongue, deeply injected and without papillæ is red, smooth and bald. The tongue may be large from swelling and show indentations of the teeth. Ulcers or erosions may occur later. In chronic nicotinic acid deficiency the first stage is redness and swelling of the fungiform papillæ, which become hypertrophied, but infiltration obscures their vessels and diminishes their redness; in the mild state the size of the fungiform papillæ is only slightly increased. In severe cases the tongue is very thick, the fungiform papillæ are markedly hypertrophied and the tongue may have a yellowish cast. The filiform papillæ on the dorsum are also markedly hypertrophied. Later in this stage infiltration of the fungiform papillæ may be complete so that they appear almost white and avascular. Later a longitudinal fissure appears down the middle of the tongue and crevices appear. The previous hypertrophy of the fungiform papillæ now gives way to atrophy; few or no filiform papillæ are on the anterior edge and margin of the tongue. As the papillæ become partly fused the tongue takes on a smooth appearance, and with final atrophy of the papillæ it becomes denuded, bald and whitish, often with a yellow cast. There may be heavy furring over the tongue, which has become thinner than in the preceding stages. Finally the lateral edges are thinned or eroded—the “slashed edges” of earlier workers. Kruse claims that these changes can be reversed by the administration of nicotinamide in doses of 200 mg. daily.

In these changes the fungiform papillæ appear to undergo change before the filiform. The same stage may not be observed all over the tongue; different sites may show different stages. The regions tend to be affected in the following order: tip, anterior and antero-lateral edges, anterior and antero-lateral margins of the dorsum, anterior border of predominantly filiform zone and mid-dorsum. In general involvement appears first anteriorly, then proceeds posteriorly. Even advanced chronic lesions disappear in the reverse sequence of their appearance, the tongue becoming thicker, regaining its vascularity, tissue substance and papillæ. According to Kruse, most cases require a very long time for complete recovery, some more than a year of daily treatment. Control groups given vitamin A or vitamin C show no improvement in their tongues.

NICOTINIC ACID THERAPY

Nicotinic acid has been used in the treatment of a number of clinical conditions, some associated with deficiency disease, while others are not. In the latter group it presumably acts pharmacologically and not as a vitamin. Nicotinamide can be used instead of nicotinic acid, and in the same dosage, in the treatment of conditions associated with defective nutrition. It is in fact preferable to nicotinic acid if the latter is to be injected or given in large doses, since nicotinic acid is liable to produce flushing and vasodilatation, which although not harmful, may prove alarming to the patient (p. 887). Nicotinamide is free from these side effects. If nicotinic acid itself is given, it is best to keep the single dose within 50 to 100 mg. to avoid the vasodilator effect. When nicotinic acid is given for its vasodilator action, *e.g.*, in coronary disease, asthma and

peripheral vascular disease, it cannot be replaced by nicotinamide, which is devoid of any such action.

Sub-clinical Pellagra, Sprue and other Deficiency States. Katzenellenbogen [91] has used nicotinic acid in doses of 50 mg. several times a day in the treatment of stomato-glossitis (endemic glossitis) in the absence of other characteristic signs of pellagra. Others have used it in the treatment of nutritional glossitis associated with leukoplakia [109] and aphthous stomatitis [107, 108, 152]. Sir Philip Manson-Bahr [89, 102] has shown that certain forms of glossitis and stomatitis are curable with nicotinic acid in daily doses of 150 mg. He also describes the treatment of patients with glossitis, aphthous ulceration, salivation and loss of taste with nicotinic acid [108]. According to Sir Philip Manson-Bahr there are reasons for believing that pellagrous glossitis is by no means rare in England among patients suffering from intestinal derangements and associated dietetic restrictions. He has treated a number of cases of tropical sprue with nicotinic acid, and states that it has a most striking effect on the glossitis, œsophagitis, and the loss of taste, which exert a profound effect upon appetite, digestion and assimilation. The fiery redness of the tongue in advanced sprue tends to fade within a day of giving nicotinic acid in doses of 150 to 800 mg. a day, and the appearance of the tongue is restored to normal in three to four days. Within four days the diarrhœa disappears and the stools are of normal size and colour in two to three weeks. If the state of the tongue reflects the condition of the entire gastro-intestinal tract, then nicotinic acid should also exert a similar action on the gastro-intestinal mucosa and influence the absorption from the small intestine, to each of which the main signs and symptoms of sprue may be attributable. According to Sir Philip Manson-Bahr strict dietary precautions are unnecessary with nicotinic acid therapy, and considerable improvement is shown in the general appearance, mentality and capacity of the patients. Flatulency and meteorism, which are persistent and distressing aftermaths of sprue, were strikingly absent from Sir Philip Manson-Bahr's cases. He advises the continued administration of nicotinic acid in doses of 150 mg. a day for at least three months, and after this a course for fourteen days of the month for six months. He also states that the harsh, dry, scaly and inelastic skin of sprue, which resembles old parchment, rapidly improves in texture under nicotinic acid therapy. He claims that no other method of treatment has given such good results in the treatment of sprue as nicotinic acid. Others have observed improvement in cases of sprue and idiopathic steatorrhœa after giving nicotinic acid in doses of 50 to 500 mg. a day [104, 105].

Vedder [236] states that the erythrocyte maturing factor of the Cohn fraction of liver relieves the macrocytic anæmia of sprue, but has no effect in controlling the intestinal symptoms, although crude liver extract has. He believes that the substance in liver extract that controls intestinal absorption in sprue is nicotinic acid.

Typhoid fever among natives often precipitates deficiency symptoms such as orolingual ulceration and mental symptoms characteristic of nicotinic acid deficiency—mania, confusion, delirium and melancholia. The restricted food intake and diarrhœa result in a deficiency state.

Sarma [287] claims to have controlled these symptoms in typhoid patients with 50 mg. of nicotinic acid a day orally. While such therapy seems rational, better results would probably be obtained by giving a daily dose of 500 mg. orally, or if absorption seems doubtful, a smaller dose given parenterally.

Bennett and Hardwick [106] discuss a clinical state designated as "chronic jejuno-ileal insufficiency," in which glossitis, steatorrhœa, emaciation, meteorism, megalocytic anæmia, hypocalcæmia and tetany result from the failure of the small intestine to perform its normal functions. They have shown that some of these symptoms react to nicotinic acid therapy.

Cases of macrocytic anæmia associated with pregnancy, pellagra and steatorrhœa and other conditions and refractory to parenteral liver, but yielding to whole liver and yeast have been reported. Ahmad [284] states that many cases of this type studied by him responded to nicotinic acid as well as to whole liver or yeast. He attributes the effectiveness of the latter to their nicotinic acid content.

Oral Conditions. "Trench Mouth." Saphir [110] has used nicotinic acid in doses of 50 mg. three times a day in the treatment of a case of xerostomia of long standing. Vincent's organisms are commonly found in the buccal lesions of pellagra and disappear with nicotinic acid therapy. King [111, 151, 289] has attempted to demonstrate an association between nicotinic acid deficiency and the syndrome variously described as "trench mouth," Vincent's disease, ulcerative gingivo-stomatitis and fusospirochætal stomatitis. The condition is characterized by the formation of a pale yellowish-grey epithelium progressing to ulceration on the gingivodental margins. It is accompanied by bleeding from the diseased capillaries, soreness and fœtor oris. King believes that nicotinic acid deficiency, reduced tissue resistance (colds, infections), chronic gingivitis, and oral trauma are contributory factors in the aetiology of trench mouth. He distinguishes three types: (a) *fulminating*, with rapid onset, occurring sporadically in spring and summer; (b) *subacute non-ulcerative* type, with a comparatively high incidence throughout the year; (c) "*flaring*" *subacute*, resembling (a) in symptomatology, but differing in that there is a history of subacute disease. Nicotinic acid in doses of 200 mg. daily, with a maintenance dose of 100 mg. for seven to fourteen days was stated to be effective in the fulminating type, but less satisfactory in the subacute and "flaring" types. King states that he found the best treatment to be local application of hydrogen peroxide and chromic acid and nicotinic acid by mouth. Vitamin C therapy was disappointing. Jacobs [240] considers that most cases of trench mouth are due to a deficiency of the vitamin B complex and vitamin C. He states that most cases clear up when given nicotinic acid, riboflavin and vitamin C. Good results with nicotinic acid therapy in the treatment of trench mouth are also claimed by Miller, Greenhut and Roth [248], Schwartzman and Grossman [244] and Smith [245].

These views have been criticized by other workers. Ungley and Horton [241] noted that eighty-five per cent. of a group of British naval ratings suffered from trench mouth, but they were unable to relate the

incidence of this to nicotinic acid or vitamin C deficiency. Nicotinic acid, 500 mg. daily, caused no improvement. Cuthbert and Williams [242] and Stammers [259] also found no evidence of nicotinic acid deficiency in trench mouth nor did they find that nicotinic acid without local treatment had any effect on the course of the disease. Stammers' conclusions were based on a study of over 1,000 cases.

Coulson, Ellinger and Smart [288] examined the nicotinamide methochloride excretion after a test dose of nicotinamide, which is considered to be an index of nicotinic acid nutrition (p. 412), in a number of R.A.F. personnel with normal gums and found it to be higher than in those with various types of gingivitis. The difference was statistically significant, but was not considered to be of ætiological importance, since other factors have a marked influence on the results. Among subjects from the same economic or social class with similar feeding habits and similar diet the excretion of nicotinamide methochloride after a test dose of nicotinamide was the same whether gingivitis was present or not.

Cardiovascular Diseases. It has been suggested that the vasodilator action of nicotinic acid might be utilized in the treatment of peripheral vascular disease, especially in extremities with a diminished blood supply [68, 112, 118, 187]. The effective oral dose is in the region of 100 to 800 mg. or 20 to 25 mg. intravenously. Whatever favourable effects it might have, they are of short duration. Loman [187] and his colleagues have shown that nicotinic acid cuts short a Raynaud attack produced artificially by adrenaline injected into the brachial artery. Green and Salber [154] state that a considerable improvement occurred in a case of hemiplegia treated with 150 mg. of nicotinic acid three times daily for fifteen days, and Furtado [246] claims that a dose of 50 to 200 mg. daily gave considerable relief in a case of cerebral thrombosis. As only single cases were reported and both conditions often result in natural recovery, it is difficult to comment on this form of treatment.

Moncrieff [162] has used nicotinic acid with good results in angina pectoris. Neuwahl [247] found that the administration of nicotinic acid by mouth decreased the severity and number of attacks of angina pectoris in a number of cases, but in some the effect was only transient. He, therefore, gave it in the form of an intravenous drip of a 0.05 per cent. solution in isotonic saline. One infusion of 100 to 800 mg. produced beneficial results, which were maximal in twelve to twenty-four hours. In most cases a course of six infusions spread over three weeks was given. Six cases showed complete or almost complete regression of symptoms over a period of three to seven months after treatment. The nicotinic acid was stated to produce a fall in blood pressure and slowing of the heart. According to Stokes [260] the changes in the electrocardiogram of cardiac ischæmia in man following the administration of nicotinic acid suggest that it can improve the coronary blood flow (Fig. 106). This only occurs after giving doses large enough to produce peripheral flushing. In a controlled clinical trial Stokes was unable to confirm the beneficial effect reported by others of nicotinic acid in the treatment of angina. In ten cases of angina that were all relieved and prevented by glyceryl trinitrate

only three received slight benefit from the administration of nicotinic acid in doses of 200 mg. daily (50 mg. q.d.s.).

Sydenstricker [288] believes that arteriosclerosis produces nicotinic acid deficiency by means of polyuria. Any factor increasing diuresis washes out the water soluble vitamins from the body (*cf.* vitamin B₁,

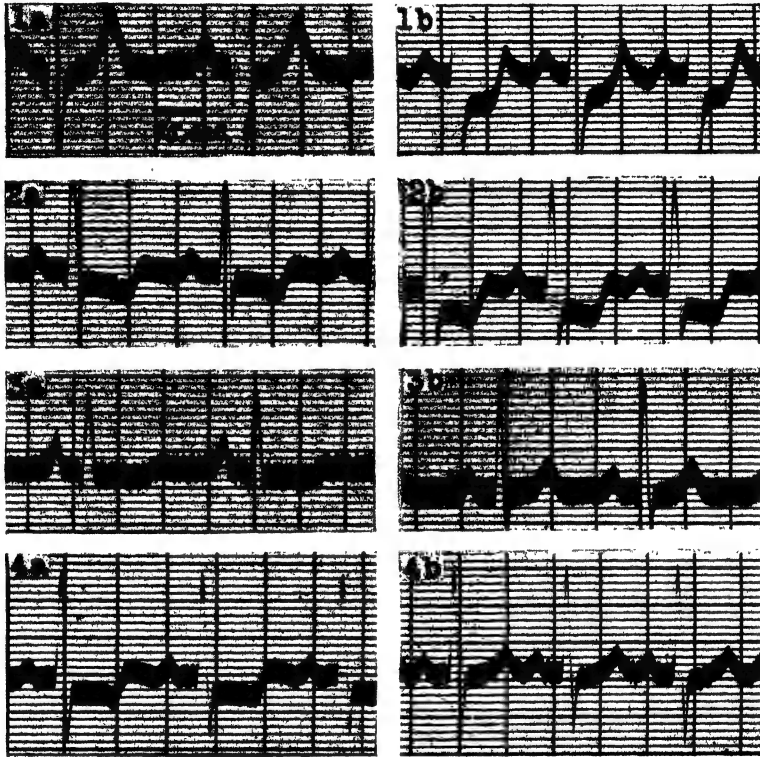


FIG. 106. Electrocardiograms in a Patient with Cardiac Ischæmia, after Administration of Nicotinic Acid and Glyceryl Trinitrate.

- (1a) At rest. (1b) After exercise inducing pain.
 (2a) At rest. (2b) 25 minutes after 200 mg. of nicotinic acid which failed to cause flushing.
 (3a) At rest. (3b) 24 minutes after 300 mg. of nicotinic acid, which caused flushing.
 (4a) At rest. (4b) 4 minutes after chewing glyceryl trinitrate gr. 1/100.

p. 212). He therefore suggests the administration of nicotinic acid in this condition.

Skin Diseases. In view of the action of nicotinic acid on the dermatitis of pellagra it was hoped that it might be effective in the treatment of some skin diseases. There are isolated reports on its value in the treatment of seborrhœic eczema [115], lupus erythematosus [116] and pruritus vulvæ [117]. Dabney [117] used it in doses of 100 mg. three times a day for the treatment of idiopathic pruritus vulvæ, and although half the cases showed improvement, he was careful to state that all local causes of pruritus

EFFECT OF NICOTINIC ACID ON SKIN LESIONS IN DIABETES

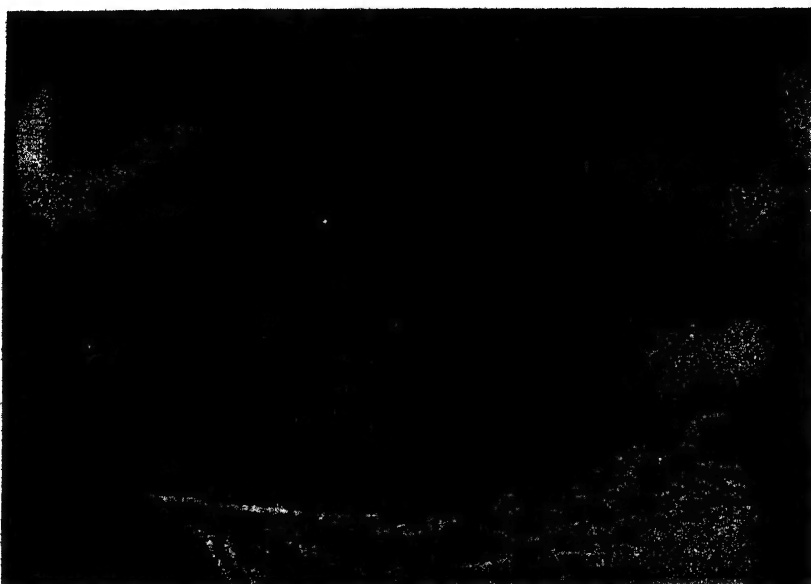


FIG. 107. Diabetic with scaly, red, indurated and dry skin lesions before treatment.

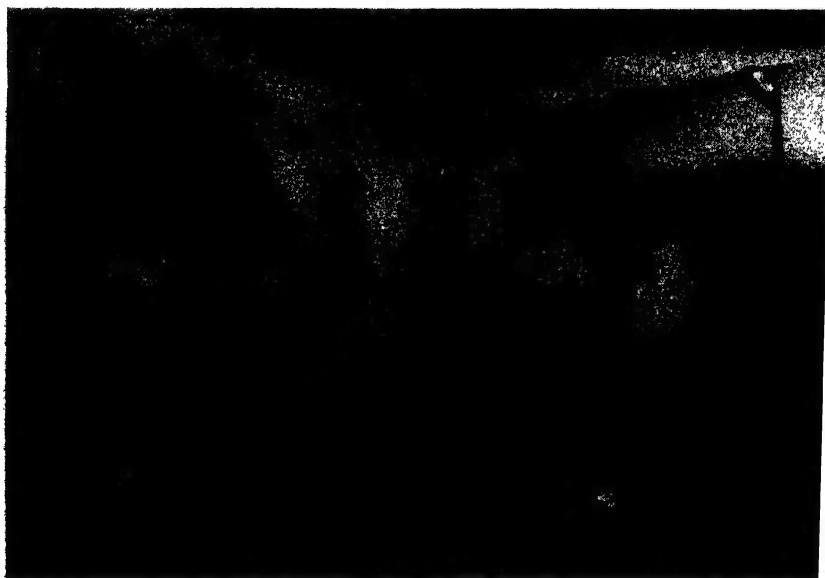


FIG. 108. Same patient as in Fig. 107 after treatment with nicotinamide 250 to 450 mg. daily. No local treatment was given and the diabetes was uncontrolled.

EFFECT OF NICOTINIC ACID ON SKIN LESIONS IN DIABETES

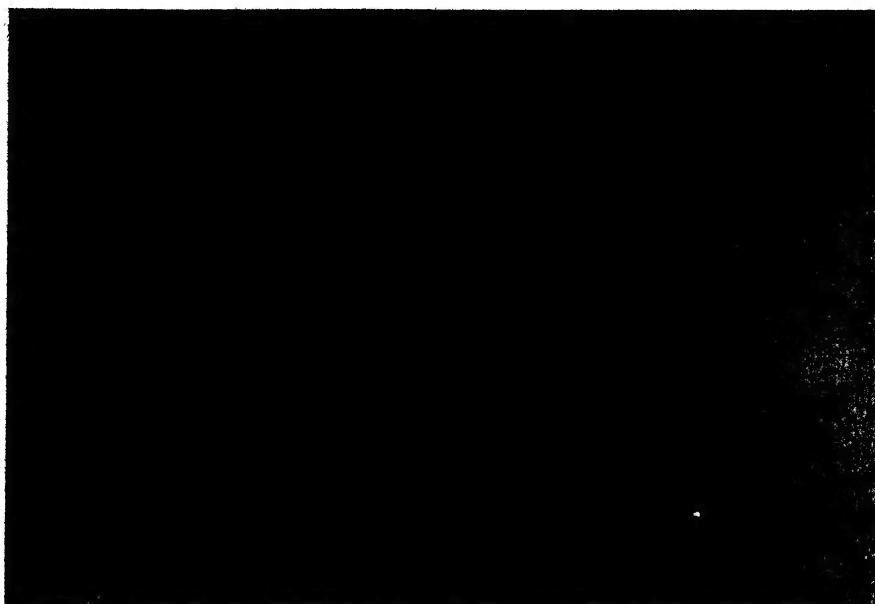


FIG. 109. Diabetic with red, indurated, dry and scaly skin eruption of the ears of three years' duration. There were deep fissures behind the ears and lesions on the breasts as in Fig. 107, and in the pubic, intergluteal, sacral and olecranon regions.

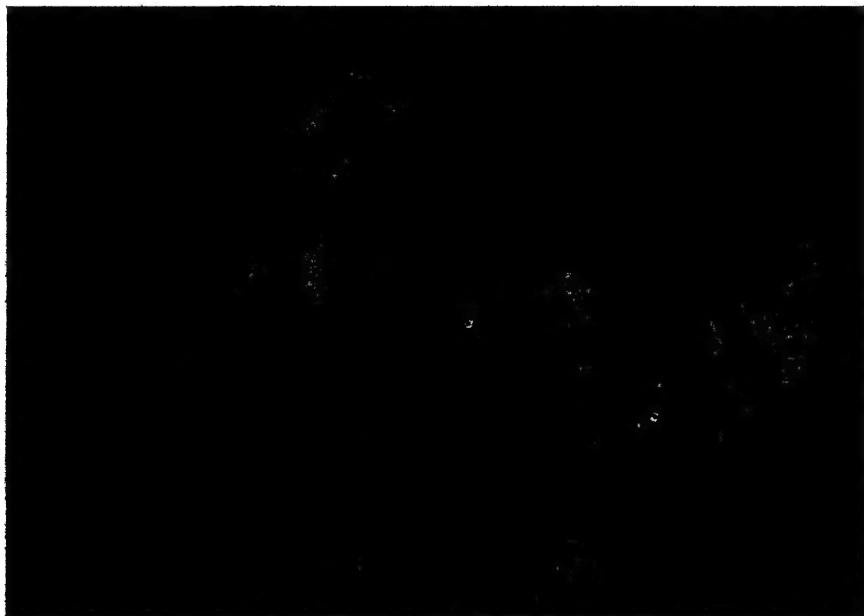


FIG. 110. Same patient as in Fig. 109 after treatment with nicotinic acid 200 mg. daily. The diet and dosage of insulin remained unchanged.

should be excluded before treatment with nicotinic acid. It is possible that improvement in these skin conditions was due to local vasodilatation and improvement in the blood supply. Greenberg [248] showed that the urinary excretion of nicotinic acid was within normal limits in a number of patients with various dermatoses. Tisdall, Drake and Brown [114] treated some cases of acrodynia with nicotinic acid on account of the superficial resemblance between pellagra and acrodynia, but they observed no demonstrable improvement on nicotinic acid alone. Birkhäuser [249] treated ten patients suffering from chilblains with 50 mg. of nicotinamide twice daily for several weeks and reported promising results. It is difficult to understand what effect the nicotinamide had, as it is devoid of any vasodilator action.

Diabetes. Neuwahl [178] noted a well-marked temporary improvement in the carbohydrate tolerance of diabetics being treated with nicotinic acid for vascular disease. He states that further investigation on a group of twelve diabetics showed that the administration of nicotinic acid or nicotinamide diminished the requirements of insulin needed to keep the blood sugar of the diabetics within normal limits. The dosage was of the order of 500 mg. three to five times daily to begin with, the dose being subsequently reduced as the blood sugar came down. The nicotinic acid and nicotinamide were given in enteric coated tablets.

Skin disturbances such as pruritus, dermatitis and intertrigo are common in diabetics, and owing to dietary restrictions some degree of avitaminosis may result. Rudy and Hofmann [250] state that these skin disturbances are most frequently due to vitamin deficiencies, particularly of nicotinic acid, rather than to disturbed carbohydrate metabolism as was formerly thought. Pellagrous dermatitis in diabetes is often seen and is sometimes diagnosed as psoriasis. Rudy and Hofmann have cured the skin lesions in a number of diabetics by the administration of nicotinic acid or nicotinamide (Figs. 107-110). They state that complete cure may take from a few days to a few months, and that more stubborn cases require large doses parenterally as well as orally. The dosage given was from 150 to 800 mg. daily in divided doses. The vitamin B complex was also given in the form of yeast.

Asthma. There are three published reports on the use of nicotinic acid in the treatment of asthma. Maisel and Somkin [251] first published a preliminary report on the treatment of asthmatic attacks with nicotinic acid. Severe attacks were controlled by the slow intravenous injection of 100 mg. of nicotinic acid, which was stated to produce improvement lasting for three to fifteen hours. Some chronic patients were improved by oral medication, 200 mg. three times daily and on retiring. The patients noted flushing after administration of the nicotinic acid followed by the expulsion of tenacious mucous plugs. The beneficial effect was attributed to a vasodilator effect on the blood vessels or relief of bronchospasm. Neuwahl [252] noted improvement in four cases, but three were made worse. Melton [253] gave nicotinic acid in doses of 50 to 100 mg., usually intravenously, to nineteen cases of asthma during acute paroxysms and obtained definite improvement in sixteen cases; two had marked exacerbations. The tests were controlled by injections of sterile water.

Nicotinic acid was also given over a long period in doses of 50 mg. two or three times daily and 100 mg. at night to thirty cases and the frequency of attacks was stated to have been reduced in sixteen. Relapse occurred after discontinuing the nicotinic acid. At present nicotinic acid can only be regarded as an adjuvant in the treatment of asthma. The fact that it makes some cases worse should be borne in mind.

Neurology. Selfridge [123, 124] has used nicotinic acid, nicotinamide and sodium nicotinate in the treatment of some thirty cases of high tone deafness, and in many the results were said to be striking. He believes that an underlying nutritional deficiency explains the nerve changes, and all cases observed by him gave a history of faulty diets. He states that both vitamin B₁ and nicotinic acid give a response in the hearing curve, but that the greatest improvement came from the use of nicotinic acid.

Harris and Moore [125] have described the treatment of twenty cases of Ménière's syndrome with 250 mg. of nicotinic acid and 20 mg. of vitamin B₁ a day; seventeen became entirely free from vertigo. They point out, however, that treatment may have to be continued for several months to obtain complete relief. Atkinson [118, 119, 161, 280] has also used nicotinic acid in the treatment of certain types of Ménière's syndrome. He states that patients suffering from this condition may be divided into two groups by an intradermal histamine test. There is a small group sensitive to histamine that can be treated by desensitization with the latter, and a large group, insensitive to histamine, the attacks being the result of primary vasospasm. In this group Atkinson found that relief could be obtained by vasodilator drugs, the most satisfactory of which was nicotinic acid. He gives an initial dose of 80 mg. intramuscularly, and if this is tolerated a dose of 25 to 30 mg. intravenously. This is repeated daily or every second day for six to eight doses, increasing the dose by 5 mg. each time to the maximum tolerated, which is usually 50 mg., but may be as high as 75 mg. After a few days oral treatment is started as well, usually 50 mg. two or three times daily. Following intravenous therapy, intramuscular therapy is started and given daily for one to three months, and then successively decreased to five, four, three, two and one administration a week. At the same time 100 to 150 mg. are given daily by mouth. After several months' treatment oral therapy alone is tried. Atkinson claims that of one-hundred-and-ten cases he has treated relief or improvement of vertigo, tinnitus and deafness occurred in eighty-four, fifty-two and twenty-three per cent. of the cases respectively. These figures are much the same as those obtained by other methods of treatment, *e.g.*, dehydration and low salt diet. Atkinson believes that Ménière's syndrome is due to anoxæmia of the labyrinth caused by vasospasm and that treatment with vasodilator drugs, such as nicotinic acid, is rational. Migraine and tinnitus aurium are stated to respond [296, 297].

The surgical treatment of trigeminal neuralgia is not completely devoid of risk and any medical method of treatment is worth investigation. Adams and Robinson [120] of Leeds, have reported that the paroxysms of the disease can be relieved by nicotinic acid in doses of from 50 mg. twice daily to 75 mg. four times daily. Furtado and Chicorro [254] also used nicotinic acid because they had observed that the pain of trigeminal

neuralgia is often accompanied by vasomotor changes in the trigeminal area. Their method is to give a daily intravenous injection of 100 to 200 mg. In four cases out of eight a few injections gave relief lasting for months, and when an injection was given during a paroxysm the relief of pain was immediate.

Moore [126] has treated cases of disseminated sclerosis with injections of 60 to 160 mg. of nicotinic acid and 33 mg. vitamin B₁ two or three times weekly. Considerable subjective and objective improvement occurred in all patients, and he attributes it in part to the vasodilator action of nicotinic acid improving the blood supply to the brain and spinal cord, thereby intensifying the action of vitamin B₁. Loman [187] and his colleagues, however, state that nicotinic acid is a relatively ineffective cerebral vasodilator, since it fails to influence the cerebrospinal fluid pressure and does not dilate the retinal vessels, which are comparable to the pial vessels.

Nicotinic Acid and Sulphonamide Therapy. The biochemical antagonism between sulphonamides and *p*-aminobenzoic acid is now well known (p. 182). Evidence is now accumulating that there is a similar antagonism between certain sulphonamides containing the pyridine ring, such as sulphapyridine, and nicotinic acid, which is β -pyridine carboxylic acid (p. 375). This is another example of the condition postulated by Fildes of two compounds with a similar chemical configuration competing for the same enzyme system. Before this view was put forward nicotinic acid was used empirically to diminish the unpleasant side effects of the sulphonamides, such as skin photosensitization, cyanosis, nausea, vomiting, depression, headache, dizziness and confusion. McGinty [127] and Doughty [129] claimed that nicotinic acid in doses of 20 to 50 mg. three times a day relieved many of these unpleasant effects produced by sulphanilamide. This is difficult to understand on Fildes' theory, because structurally sulphanilamide and nicotinic acid have nothing in common. It has also been observed that whereas sulphapyridine can block nicotinic acid or its amide as growth factors for bacteria, sulphanilamide cannot [179]. On theoretical grounds it is reasonable to suppose that clinically nicotinic acid can relieve the side effects produced by sulphapyridine, but it must also be realized that in concentrations necessary to do this it may inhibit the bacteriostatic action of the drug. Schaefer and his co-workers [255] gave sulphapyridine to dogs with nicotinic acid deficiency to produce a blood level of 5 to 10 mg. per cent. Nicotinic acid, nicotinamide and dried liver extract caused rapid improvement in some of the local lesions, but did not correct the loss in weight and anorexia. Brown and his colleagues [128, 256] claim that nicotinic acid in doses of 50 mg. decreased the nausea and vomiting due to sulphapyridine in about eighty-five per cent. of their cases. Nicotinic acid does not prevent the anaemia resulting from large doses of sulphonamides [182].

Some of these clinical observations were made in the early days of sulphonamide therapy and need confirmation.

As it is possible that considerable amounts of nicotinic acid are synthesized by bacterial flora in the human gut, and that this synthesis is inhibited by the bacteriostatic action of the sulphonamides [265, 266], it

is advisable when giving these drugs to administer supplements of nicotinic acid and other B vitamins.

Other Uses. It is claimed that nicotinic acid relieves the toxic effects due to poisoning with heavy metals such as thallium [184] and lead [188, 257]. Graham [185] has used nicotinic acid in the treatment of seventy patients undergoing X-ray therapy. Only those suffering from severe nausea and vomiting were given nicotinic acid in doses of 80 to 200 mg. three times a day. The results obtained were stated to be better than with other forms of treatment. Similar claims are made by Kepp [181], who treated his cases with 500 mg. of nicotinamide daily. Bean, Spies and Vilter [279] state that patients on diets poor in the vitamin B complex readily develop irradiation sickness, which can be prevented or relieved by administering nicotinic acid or vitamin B₁ a few days before exposure. Well-fed patients had little reaction to the same dose of X-rays that made patients deficient in the vitamin B complex sick. The same authors record a case of classical beriberi and pellagra developing after irradiation therapy. They suggest that the basic disorder in irradiation sickness is a disturbance in the respiratory enzyme systems, of which nicotinic acid, vitamin B₁ and other members of the vitamin B complex are components. The excretion of urinary pigments and codehydrogenases I and II after irradiation therapy is similar to that observed in pellagrins. As the diet is often already deficient in patients needing radiotherapy, it would appear rational to supplement it with the vitamin B complex before commencing treatment.

Perdue [258] has used nicotinamide in doses of 100 mg. in labour to prevent the depressant effects of barbiturate-hyoscine narcosis on the respiratory centres of mother and child. He states that anoxæmia in the mother and apnoea in the newborn are minimized by this procedure. Although one hundred and fifty-nine cases were studied, there were no controls.

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CHAPTER VI

VITAMIN C

(ASCORBIC ACID)

HISTORY

SCURVY has been a menace to seafarers, explorers, and armies since classical times, although the first recorded accounts of the disease are to be found among the writings of the physicians who accompanied the crusaders in the thirteenth century. The decline of scurvy in Europe coincides with the introduction of the potato in the early seventeenth century and the increased consumption of vegetables and fruit. The havoc caused by the disease among sailors is described in many a ship's log. Thus Vasco da Gama, who sailed round the Cape of Good Hope in 1498, describes how a hundred of his crew of a hundred and sixty perished from scurvy at sea. Sir Richard Hawkins' "Observations in his Voyage to the South Sea" (1598) contains an account of the prevention and treatment of scurvy with lemon juice. Jacques Cartier (1535) during his exploration of Canada found that the native Indians prevented the disease by drinking a decoction of pine needles, a preparation that has recently been used in Scandinavian countries as an antiscorbutic remedy.

Lind in his famous book, "A Treatise on the Scurvy" (1757), mentions the value of fresh citrus fruits and green vegetables in the treatment of scurvy. He gave patients suffering from the disease various forms of treatment, including two oranges and a lemon a day. Only those receiving the oranges and lemons showed any improvement. During his voyage round the world between 1772 and 1775 Captain Cook kept his men free from scurvy by including in their dietary as much fresh food as possible, including fruit and vegetables. In 1804 regulations were introduced into the British Navy providing all ratings with daily rations of lemon juice, and similar provisions were made by the Board of Trade in 1865.

To-day frank scurvy is almost a disease of the past. It is still met with, however, in isolated parts of the world and during wartime. During the war of 1914-1918 outbreaks occurred among British troops at the siege of Kut, and several cases occurred among civilians in Glasgow, Newcastle and Manchester owing to a shortage of potatoes.

Modern nutritional work on scurvy dates from 1907, when Holst and Frölich of Christiania tried to produce beriberi in guinea-pigs by means of restricted diets, but scurvy resulted instead. Henceforth the guinea-pig was used in all experimental work on scurvy. After Funk's postulation in 1912 of a scurvy-preventing or antiscorbutic vitamin, vitamin C, attempts were made to isolate it from orange and lemon juice. Between 1924 and 1929 Zilva [1] and his co-workers succeeded in obtaining a concentrate from lemon juice, 1 to 2 mg. of which protected guinea-pigs from scurvy if given daily.

In 1928 Szent-Györgyi actually isolated vitamin C from cabbage and adrenals without knowing it during the course of studies on cellular

oxidation. He called it "ignosic" acid because it resembled a sugar that he called "ignose." When the editor of the journal to whom his report was sent objected to "ignose," Szent-Györgyi altered it to "Godnose." This was equally objectionable to the editor and the name was finally altered to "hexuronic acid," which was later identified with vitamin C. "I became a father," said Szent-Györgyi, "without wishing it, the father of a vitamin. Such accidents seem to happen in science" [596].

In 1932 Waugh and King [2] isolated vitamin C from lemons and identified it with the "hexuronic acid" of Szent-Györgyi, who reported that a daily dose of 1 mg. protected guinea-pigs against scurvy [3]. In the same year Waugh and King [4] showed that the antiscorbutic activity of hexuronic acid was identical with that of the vitamin C obtained from orange juice.* The identity of these two substances was further demonstrated by Harris [5] and other workers. It is of particular interest to note that Tillmans and his associates pointed out the close association between the antiscorbutic value of foodstuffs and their quantitative reduction of the indicator 2 : 6-dichlorophenolindophenol, which is now largely used for the estimation of vitamin C (p. 548).

The structural formula of vitamin C was established in 1933 as a result of the work of Haworth, Hirst and their co-workers [6], Karrer [7], and a number of other investigators. In the same year Reichstein [8] and his colleagues in Switzerland synthesized the *d*- and later the *l*-form of the vitamin. Almost simultaneously Haworth and Hirst [9] synthesized it in this country. It is now prepared by an improved method due to Reichstein and his collaborators [11]. In 1933 Szent-Györgyi and Haworth [10] suggested the name *ascorbic acid* for vitamin C to denote its antiscorbutic action. The Council on Pharmacy and Chemistry of the American Medical Association adopted the name *cevitamic acid*, and although this was used in the literature for some years it was finally abandoned in 1939 in favour of the name ascorbic acid [597].

CHEMISTRY OF VITAMIN C

Vitamin C forms colourless crystals melting at 190° to 192° C., with a specific rotation of + 23° in water and + 48° in alcohol. It is freely soluble in water, and slightly soluble in acetone and the lower alcohols. It is insoluble in benzene, ether, chloroform and fats. There is no carboxyl

* There is some doubt whether there is another factor besides vitamin C with an antiscorbutic action in fruit and green vegetables.

Thus there are clinical cases of scurvy that have not responded to pure vitamin C, but have to orange juice [86]. Todhunter *et al.* [87] report that the antiscorbutic value of a given quantity of lemon juice is greater than that of an equivalent quantity of vitamin C. They consider that lemon juice may contain an additional factor with an antiscorbutic action. Agnew *et al.* [87] state that a deficiency of vitamin C in guinea-pigs cannot be entirely corrected by administering synthetic vitamin C, but only by feeding fresh cabbage. It is also reported that the vitamin C of raw cabbage and tomato juice may be utilized better than synthetic vitamin C [881]. On the other hand there are several studies in human beings and animals showing that the urinary excretion and blood plasma level are practically identical in any one subject or animal when vitamin C is administered either as the synthetic vitamin or in fresh fruit and vegetables [88, 78, 811, 1037].

group in the compound, salts being formed by the dissociation of an enolic hydrogen atom. Vitamin C condenses with aldehydes, acetone and other ketones in the presence of mild dehydrating agents to form stable crystalline derivatives.

The reactions of greatest interest and importance are those dealing with oxidation and reduction. If kept dry and not exposed to light vitamin C is stable for a considerable time. Tablets turn slightly yellow on keeping, but even after two years in tropical climates there is practically no loss of potency as tested by the indicator 2 : 6-dichlorophenolindophenol. Visible light and ultra-violet light have a markedly destructive effect on the vitamin, which is hence stored in yellow-coloured bottles.

In solution the stability of vitamin C depends upon many factors.

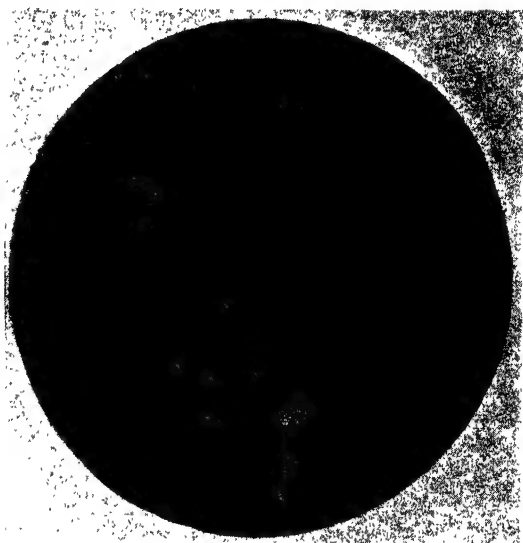
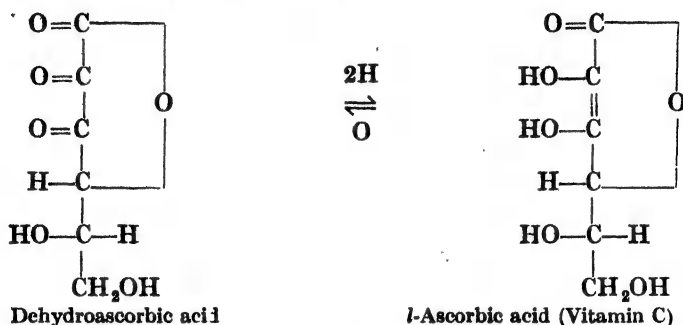


FIG. 111. Crystals of Vitamin C.

The presence of iron and copper ions rapidly catalyses the oxidation of the vitamin ; copper is active even in a concentration of $20\mu\text{g}$ per litre. Vitamin C is stable in the presence of aluminium and stainless steel. In aqueous solutions below $\text{pH } 7.6$ vitamin C is not oxidized on exposure to air unless traces of copper or other such catalyst are present [12]. But in the presence of oxygen destruction is rapid and complete when the pH is above 7.6 and heat is used. Autoclaving at 120°C . for twenty minutes in oxygen at $\text{pH } 8$ results in a loss of forty-nine per cent. ; in carbon dioxide and nitrogen only twelve per cent. at $\text{pH } 8$, and four per cent. at $\text{pH } 3.2$ [13]. It has been found that solutions of vitamin C can be stabilized by the addition of small amounts of fruit acids such as tartaric and citric acids. In the plant, factors are present inhibiting the oxidation of vitamin C [882]. Numerous substances such as tissue extracts, glutathione, cystein [883], allyl isothiocyanate [884], thiourea [885], carbon monoxide, cyanides and

halide ions [868], all of which form compounds of a low degree of ionisation with copper, inhibit the oxidation of vitamin C. Copper is a component of ascorbic acid oxidase, which slowly oxidizes vitamin C in fruit and vegetables after ripening or after gathering (p. 450). Solutions of vitamin C and its sodium salt are quite stable if kept in air-free vessels or in an inert atmosphere of nitrogen or carbon dioxide. The first oxidation product of vitamin C is dehydroascorbic acid. The oxidation of vitamin C to the latter is reversible by hydrogen sulphide, cysteine and glutathione.



Dehydroascorbic acid is as potent an antiscorbutic as ascorbic acid itself.

Vitamin C is reversibly oxidized by a number of organic compounds such as methylene blue, quinones and indophenol dyes, which are used in its estimation. Aqueous iodine also oxidizes it, two atoms of iodine reacting with one of the vitamin. In the presence of alkali iodine quantitatively oxidizes vitamin C to oxalic and trihydroxybutyric acids.

Estimation of Vitamin C. The method commonly in use for the estimation of vitamin C in foodstuffs and body fluids is by titration against the blue dye 2 : 6-dichlorophenolindophenol, the colour of which is discharged by vitamin C. Details are given on p. 543. The method is not specific, as sulphhydryl compounds, thiosulphate, pyridinium compounds, nicotinic acid derivatives, riboflavin, and iron compounds interfere with the estimation. Other reducing compounds behaving like vitamin C are found in foodstuffs. A certain amount of combined vitamin C and dehydroascorbic acid are found in foods and body fluids; these are not determined by the indophenol reaction. Before estimation, dehydroascorbic acid must be converted to vitamin C by hydrogen sulphide. The vitamin C in coloured fluids can be estimated using a photoelectric colorimeter [586, 887, 888]. Extraction or preservation of the material with glacial acetic acid, trichloroacetic acid or metaphosphoric acid helps to prevent the oxidation of vitamin C. The specificity of the determination of vitamin C by indophenol has been increased by adding formaldehyde to the fluid containing vitamin C [889]. Vitamin C does not reduce 2 : 6-dichlorophenolindophenol in the presence of twenty per cent. hydrochloric acid [985]. This affords a method of estimating it in the presence of interfering substances, which are reduced under these conditions.

The estimation of vitamin C in foodstuffs by chemical means is complicated by the fact that reductone is produced from hexoses when foods

are heated in alkaline solution and reductic acid is formed from pectins and pentoses in acid solution. Both these substances may interfere with the estimation of vitamin C by the indophenol method, which may therefore give erroneous results except in expert hands.

Vitamin C reduces methylene blue in the presence of light to a colourless compound. This reaction has frequently been used to estimate vitamin C [583, 584].

Other methods of estimation depend upon: (a) the conversion to dehydroascorbic acid, which reacts with 2:4-dinitrophenylhydrazine to form a red compound which can be estimated by the photoelectric colorimeter [860]; (b) the formation of a coloured compound with silicomolybdic acid [890]; (c) the reducing effect on ferridipyridyl sulphate, the coefficient of absorption of the ferrodipyridyl compound at 510 m μ being measured with a spectrophotometer.

A new bioassay method of estimation has been described based on the increase in serum alkaline phosphatase of scorbutic guinea-pigs observed after a critical dose of vitamin C [891].

UNITS OF VITAMIN C

Before the identification and isolation of vitamin C preparations containing the vitamin were biologically assayed by observing their curative antiscorbutic action on the guinea-pig, judged either by the protective action on the teeth or by its ability to prevent loss of weight. At the League of Nations Conference on Vitamin Standards in 1931 the fresh juice of the lemon was adopted as an international standard, 1 unit of vitamin C being defined as that amount of the vitamin possessing an antiscorbutic activity of 0.1 c.c. of fresh lemon juice. This is about one-tenth of the daily dose necessary to prevent the development of the macroscopic scorbutic lesions in a young guinea-pig on a scurvy-producing diet. As vitamin C is used in the form of a pure chemical substance and can be assayed chemically, the potency of any preparation or food is not expressed in terms of units but in milligrams of the actual vitamin. The international unit of vitamin C is equivalent in antiscorbutic activity to 0.05 mg. of pure *l*-ascorbic acid; 1 mg. of vitamin C is therefore equivalent to 20 I.U. The international unit of vitamin C has now disappeared from medical and scientific literature.

DISTRIBUTION OF VITAMIN C IN FOODS

Vitamin C appears to be present in all living tissues, but fresh fruits and plants are the best sources. Among the richest sources are rose hips and haws, black and red currants, strawberries, "greens" and the citrus fruits. It was formerly supposed that the citrus fruits were the most potent sources of the vitamin, but since chemical methods have become available for its estimation it has been shown that this is by no means so. Black currants, for example, are three to four times as potent as orange juice. It has also been found that the major portion of the vitamin C in the orange is located in the peel and not in the juice. Other fruits vary

considerably in their vitamin C content. Thus plums, pears and melons contain negligible quantities, while potatoes, spinach, cabbage, watercress and turnips contain relatively large amounts. Among the majority of the population the potato is one of the most important sources of vitamin C. Half a pound supplies about 80 mg. of vitamin C a day, which is enough to protect against scurvy and supplies the major portion of the daily requirement of the vitamin. Two-thirds of an ounce of black currants will also suffice for the day's ration.

The richest source of vitamin C is rose hips. Rose hips from species grown in Scotland and northern England contain more vitamin C than those grown in the south. The former contain an average of more than 1,000 mg. per 100 grams of flesh, while those from southern England contain less than 250 mg. per 100 grams. The rose hips of certain foreign roses contain up to 4,800 mg. per 100 grams [810]. Rose hips should be picked before they are over-ripe, otherwise they may lose half their vitamin C content. Rose hips are converted into syrup, which since 1942 has been issued by the Ministry of Food, with a declared potency of 175 mg. to 200 mg. per 100 c.c. It has been shown, however, that such syrup loses its vitamin C fairly quickly. In three to five months there may be a loss of fifty per cent. in the vitamin C value; in one case it was ninety per cent. [894]. A high sugar content and the presence of sulphur dioxide minimizes the destruction of vitamin C in fruit syrups on storage [1046]. Rose hip tablets are now available, and are stated to contain 1,000 mg. per 100 grams. They are very stable, losing only five per cent. of their potency in two to four months [1055]. It has been estimated that rose hips could supply all the vitamin C needed in areas in which it is endogenous. Thus the annual yield from Alberta, Canada, is sufficient to supply 140 million persons, the population of the United States, with 100 mg. of vitamin C daily [895]. Green walnuts are rich sources of vitamin C, and may contain as much as 8,000 mg. per 100 grams [880, 900].

A well-balanced diet containing plenty of vegetables and some fruit occasionally will supply the normal requirements of vitamin C for a few pence in peace time. Thus half a pound of potatoes, a helping of cabbage, and an ounce of watercress supply sufficient vitamin C, provided the foods are not spoilt in cooking and provided the cooking water is consumed as well. It has been calculated by Olliver [18] that a good helping of brussels sprouts may contain a day's requirement of vitamin C, and American workers have calculated that 100 grams (8½ ounces) of uncooked greens contain as much as 75 to 160 mg. of the vitamin [226]. Small but appreciable quantities are present in animal products. Indeed it is possible to remain free from scurvy on a diet consisting solely of underdone meat (p. 508). The richest animal sources of vitamin C are glandular tissues, particularly the suprarenals, and actively functioning tissues (corpus luteum, thyroid, liver).

The vitamin C content of cows' milk is very variable. The value drops as soon as it leaves the cow and cools down. Even in twenty-four hours raw milk may lose twenty to thirty per cent. of its vitamin C. Traces of copper in the vessels, sunlight, and pasteurization reduce the quantity still further [84]. Unless special care is taken pasteurization may result in

the loss of thirty to sixty per cent. of the vitamin C of milk. Even after the delivery of domestic milk the small residual amount of vitamin C is further decreased by keeping on the doorstep (p. 455) and by reheating. A pint of commercial milk contains from 4 to 17 mg. vitamin C; the same amount of mother's milk contains 22 to 57 mg.

It has been stated that vitamin C exists in the combined condition in certain foodstuffs and is liberated on cooking [82]. Harris and Olliver however, have found no evidence for this [17]. Wokes and his co-workers [914] have shown that the "apparent vitamin C" supposedly liberated from foods on heating is not vitamin C at all, but reducing substances such as reductone, reductic acid, dihydroxymaleic acid and hydroxytetronic acid, which, like vitamin C, reduce 2 : 6-dichlorophenolindophenol. They are formed by the action of heat on certain food constituents, *e.g.*, pectins and carbohydrates. The high vitamin C content reported for walnuts and parsley may be due to "apparent vitamin C." Generally speaking the vitamin C content of green vegetables and fruit increases to a maximum just before ripening and then decreases steadily. The content is influenced by the method of growing, the soil, the season, the time of picking, variety and the time taken to reach the table [16]. The vitamin C content is highest in late summer and autumn [898]. The vitamin C of potatoes for example is 20 to 30 mg. per 100 grams in autumn and only 5 to 7 mg. in the spring.

Variations may even be found in different fruits on the same plant or in different leaves in the same vegetable. In most fruits the outer portion contains more than the centre, although in potatoes the converse is true. Tomato skins for example contain two to three times as much vitamin C as the fleshy part [901].

Properly cooked green leafy vegetables (cabbage, spinach, sprouts, kale, broccoli, and vegetable greens such as turnip tops) are fairly good sources of vitamin C, especially as they are consumed almost daily. Apples, bananas, green peas and beans do not contain a considerable amount of vitamin C. Unlike most apples Bramley seedlings contain appreciable amounts. The raw vegetables such as lettuce and watercress are important, because they are consumed raw and all the vitamin C present is therefore available.

Dried cereals and legumes are poor sources, but if they are allowed to germinate vitamin C is formed.

Effect of Cooking [17, 18]. Until recently it was taught that most of the vitamin C in foodstuffs was destroyed on cooking, particularly in presence of alkalis. This has now been disproved [14]. It is true that pure vitamin C is rapidly inactivated when heated in solution with alkalis, but in natural foodstuffs there are stabilizing factors preventing its decomposition. During cooking a proportion of the vitamin is extracted from the tissues and passes into the cooking water so that it may not be consumed, but the actual destruction is relatively small. Cooked (not overcooked) vegetables such as cabbage, spinach, sprouts, cauliflower, together with the water they are cooked in, still retain much of their vitamin C. As soon as vegetables or fruits are gathered an enzyme known as ascorbic acid oxidase is liberated, and this slowly oxidizes the vitamin C. Ascorbic acid

oxidase is a metal protein complex, in which the prosthetic group is copper [814]. It has recently been obtained in a highly purified form [886]. Lampitt and co-workers [1045] doubt the activity of ascorbic acid oxidase and believe that the destructive mechanisms attributed to it are due to ionic copper. Fruit and vegetables should therefore not be kept too long before cooking. Cooking in iron, copper or badly "tinned" vessels rapidly facilitates the autoxidation of vitamin C. Enamel, pyrex glass, stainless steel, and aluminium do not have any deleterious effect on the vitamin. Less vitamin C is lost in the preparation of fruits and vegetables if the boiling is done rapidly with the lid of the vessel on [18, 19], and the material is plunged straight into boiling water [1045]. This rapidly destroys the ascorbic acid oxidase. Thus in rapid cooking the destruction may be as little as fifteen per cent., while in slow cooking it may be as much as thirty-six per cent. [19]. The rate of boiling is important on account of the ratio of water to vegetables at the end of the cooking. According to Olliver [18] from six to eighteen per cent. of the vitamin C of common fruits and vegetables is destroyed on cooking. Twenty to twenty-five minutes boiling in a vessel with the lid on produces no more loss of vitamin C in vegetables than seven minutes boiling [1066].

The greatest loss occurs not through its destruction, but by its extraction in the cooking water which is rejected. From twenty-five to nearly sixty per cent. may be lost in this way. Less vitamin C is lost by leaching if successive quantities of vegetables are cooked in the same cooking liquid. The continued heating or "keeping hot" of dishes causes destruction. Olliver [18] has shown that the belief that cooking vegetables with soda destroys all the vitamin C is quite unfounded; from eighteen to thirty-two per cent. is destroyed in the case of greens, figures which are not significantly different from the loss due to boiling with water. This has been confirmed by other workers [913]. Soda does, however, accelerate the decomposition of vitamin C in vegetables kept hot.

The whole of the vitamin C present in fruit is not destroyed in the preparation of jam. The amount of vitamin C in jams containing thirty to forty per cent. fruit (Food Manufacturers' Federation standard) is approximately thirty to forty per cent. of the amount in the fruit used. The cane sugar in the jam protects the vitamin C from oxidation. If vitamin C is added to jam to the extent of 0.1 to 0.4 per cent., no appreciable losses occur. The vitamin C content of jam slowly falls on keeping, *e.g.*, twenty to thirty per cent. after three months [902]. This figure depends upon the state of the fruit when picked and under optimum conditions of picking and storage, the loss in vitamin C may be as little as ten per cent. after six months' storage. A high sugar content and the presence of sulphur dioxide minimises destruction of vitamin C in fruit syrup [1046]. According to Wokes [894] the vitamin C in rose hip syrup falls rapidly on storage (p. 448). Pollard [1046] has shown that the vitamin C content of black currant syrup of high sugar content is eighty-five to ninety-five per cent. of the original even after one year's storage; after two to four years it may fall to seventy-five to eighty per cent. Wokes and Organ [1056] state that the rate of loss of vitamin C in black-currant syrup is affected by degree of exposure to air, light and storage

temperature. It should be stored in the dark in a cool place and the bottle should be used up quickly once opened.

The presence of cooking salt (quarter to two per cent.) retards the oxidation of vitamin C in foodstuffs [20].

Boiling directly and rapidly over a flame conserves more vitamin C than does steaming, but in the latter process less is extracted. If the cooking water is rejected, as it often is, steaming is therefore preferable. Olliver's figures show that a considerable amount (up to sixty per cent. or more) of the vitamin may be lost by extraction in the cooking water. Appreciable amounts of vitamin C are present in the cooking water from vegetables, as much as 80 mg. being present in 4 ounces of liquid from turnip greens [226].

In steaming, twenty-five per cent. more vitamin C is retained than when the material is boiled, except in the case of root vegetables. Baked potatoes contain approximately the same amount of vitamin C as boiled potatoes (approximately twenty per cent. loss), and weight for weight potato chips contain more vitamin C than baked or boiled potatoes, because of the considerable water loss and rapid rise of temperature [896].* Mashing potatoes and keeping them hot destroys the vitamin C rapidly, *e.g.*, from 5 to 1.8 mg. per 100 grams in twenty minutes [897]. The vitamin C of potatoes is retained better if the whole unskinned vegetable is cooked and eaten with the skin on [1068]. Other vegetables are better cooked whole than chopped up [21]. The whipping up of potatoes with air inactivates some of the vitamin C.

Stewing and baking are slow methods of cooking and therefore tend to be more destructive of vitamin C than boiling or steaming. It is doubtful whether stews containing vegetables, made on a large scale in camps, institutions and restaurants and cooked slowly for a long period, ever contain any vitamin C, unless the vegetables are added shortly before serving. Also the interval will be so long before the contents of a large stew reach boiling point that the ascorbic acid oxidase will have considerable opportunity of causing serious destruction of the vitamin C. Haybox cooking is even more destructive of vitamin C [17].

Frying is a rapid method of cooking and if the temperature is not too high does not result in the inactivation of considerable vitamin C. It rapidly seals the food, drives off much of the water, and is quick. The vitamin C content of fried foods compares favourably with that of boiled or baked [896].

Considerable destruction of the vitamin C of fruit and vegetables may occur at room temperature if the material is shredded or chopped, *e.g.*, a shredded lettuce loses eighty per cent. of its vitamin C in one minute [22]. The loss is greater if a steel knife or "chopper" is used [1047].

There is a deplorable degree of subnutrition due to the waste of vitamin C in the cooking of vegetables. "Greens" are soaked, then boiled or simmered for hours, cooked in soda, the cooking water thrown away, and meals kept hot for some time or heated up. Potatoes are thickly peeled

* Mapson (private communication) gives the vitamin C content of cooked potatoes as chips > baked > boiled > boiled and then mashed. He has noted a content of 80 mg. per cent. in potato chips.

and the outer leaves of cabbage thrown away. There is often an enormous difference between the vitamin C content of the raw material and that reaching the table [892]. If food cannot be eaten just after it is cooked it is better to allow it to cool and reheat when necessary rather than keep it hot; this conserves more vitamin C.

Much has been written on the losses of vitamin C that occur in food served in restaurants and institutions, where large quantities of food are cooked well before midday and kept hot for hours. Nagel and Harris [898] and Heller and co-workers [899] analysed a large number of restaurant and cafeteria meals and found that by the time the food reached late diners as much as seventy to seventy-five per cent. of the vitamin C was lost; the range was from twenty-nine to ninety-six per cent. Twenty per cent. was lost on cooking, twenty-five per cent. in the cooking water, and twenty-five per cent. while keeping hot. Similar figures are given for Army cooking [1054]. Losses of ninety-five per cent. in the vitamin C of potatoes have been recorded after preparation and prolonged standing on a hot plate or steam table [1068]. The following figures taken from Olliver [896] show the losses occurred in keeping cooked cabbage hot for periods from fifteen to ninety minutes.

Keeping hot 15 minutes causes destruction of 25 per cent of vitamin C.

"	"	30	"	"	"	"	40	"	"	"	"	"	"
"	"	45	"	"	"	"	50	"	"	"	"	"	"
"	"	60	"	"	"	"	60	"	"	"	"	"	"
"	"	75	"	"	"	"	70	"	"	"	"	"	"
"	"	90	"	"	"	"	75	"	"	"	"	"	"

Effect of Storing, Cold Storage, Canning, Dehydration, etc. [24-31]. Loss of vitamin C may occur when fresh foods are stored for any length of time between purchase and consumption [14, 18]. There is a rapid drop in the first three or four days [25]. Temperature, freshness, care in handling and the particular type of foodstuff are important factors. Thus spinach kept for a few days at room temperature and allowed to wilt loses half its ascorbic acid, while the same material kept at 37°-88° F. for the same period retains most of its vitamin C. In view of these results it is advisable that foods be purchased and prepared for consumption im-

Effect of Storage on the Ascorbic-acid Content of Fruits and Vegetables

Fruit or vegetable	Storage at room temperature		Storage at 32° F.	
	Period of storage after harvesting	Loss of ascorbic acid (%)	Period of storage after harvesting	Loss of ascorbic acid (%)
Black currants . . .	4 days	Nil	10 days	Nil
Gooseberries . . .	1 week	"	8 weeks	"
Greengages . . .	5 days	"	2 "	"
Peas (in pod) . . .	2 "	24	6 days	10
Potatoes (new) . . .	{ 5 "	{ 35	{ 5 "	{ Nil
	{ 2 weeks	{ 52	{ 2 weeks	{ 6
Stringless beans . . .	3 days	20	3 days	3

mediately before use. It is common practice in some kitchens to cut fruit (e.g., grapefruit, lemon) for some purpose and to keep the other half for use at a later date. This leads to loss of vitamin C. Thus the vitamin C content of an orange fell from 89.8 mg. per cent. on the day of purchase to 18.1 mg. seven days after cutting. Grapefruit appears to lose less vitamin. It has been pointed out that the systems of stabilizers and oxidases affecting vitamin C vary widely in different species of fruits and vegetables.

Olliver [896] states that for leafy vegetables and legumes there is approximately a loss of ten per cent. in the vitamin C for every day's storage. With root vegetables the destruction is not so rapid because there is less surface, but the loss may become significant because root crops may be kept for months after lifting. In the case of fruits, these keep fairly well on normal storage unless the skin is broken or the fruit has gone soft, when ten per cent. of the vitamin C is lost for each day of storage.

There is no appreciable loss of vitamin C in fruits and vegetables kept in cold storage or treated by the "quick-freeze" process, in which the food is kept from 0° to 40° F. until sold on the retail market [20]. Peas can be kept frozen for nearly a year at -18° C. with practically no loss of vitamin C. There is very little loss of vitamin C if freshly picked fruit and vegetables are packed in crushed ice and transported in refrigerated containers [1064]. Some losses do occur, however, as a result of processing operations such as shelling, washing and blanching. The latter process is necessary to destroy the enzymes which might inactivate the vitamin C during the thawing of the foodstuff. According to French and Abbott [30] the vitamin C concentration of oranges and grapefruit actually increases during the first few weeks of cold storage and then drops off slowly. The oxidation of vitamin C in orange, tomato and pineapple juice proceeds so slowly in domestic refrigerators during a period of a few days that there is hardly any loss. There is slight loss, however, in the freezing of strawberries, cranberries, red and black currants. In the case of frozen vegetables the stability of the vitamin C also seems to depend on variety, soil, climate, age, temperature, and the blanching process. Speed is essential between the harvesting of the material and the blanching. The minimum loss of vitamin C results when the blanching period is just sufficient to inactivate ascorbic oxidase and the product is cooled with all possible speed. The vitamin C in refrigerated vegetables keeps for a considerable period if the material is kept in a closed vessel [1066].

Little loss of vitamin C occurs during the defrosting of frozen foodstuffs either in a refrigerator or at room temperature. When the frozen material is cooked with a little water or steamed as much as eighty per cent. of the vitamin C may be retained [904]. When frozen peas are scalded in water, thirty to thirty-seven per cent. of the vitamin C is lost. After cooking frozen peas there is a loss of forty to fifty per cent. of the vitamin C content [908]. The vitamin C of cooked food is not retained for long, even if the material is kept cold. Thus cooked broccoli kept in a refrigerator loses ninety per cent. of its vitamin C in twenty-four hours.

Pickling, curing, salting, fermenting and the preserving of fruit pulp result in complete destruction of vitamin C.

Controlled tests have shown that the antiscorbutic value of fruits and vegetables after commercial canning is no less than that of similar foods cooked by careful household methods. In fact, canned foods are often better sources than food carelessly cooked in the household. In the canning of food the latter is freshly picked and heated under ideal conditions ; in domestic cooking the food is often bought after storage in a shop, over-cooked, and the cooking water thrown away. In the process of canning there is little destruction of the vitamin C during the cooking, which is done for a short time in vessels from which air has been extracted. About fifty per cent. of the vitamin C is in the canning liquid. Storage in the can results in a very slow deterioration of the vitamin C. Thus Daniel and Rutherford [38] found a steady loss amounting to twenty-four per cent. in one to six months' storage. The loss is negligible if the tins are stored in a refrigerator. Thus Ross found no loss of vitamin C in canned orange juice kept for a year [1068].

Contamination with the copper of the "tin," which often occurred in the past, destroys vitamin C. This has been overcome by suitably lining the tin.

From the standpoint of the housewife it is satisfactory to use tinned fruit and vegetables, although the fresh material if available is preferable. Once the can is opened the vitamin C is fairly stable for a few days if the material is kept covered in a refrigerator [1062].

Recently, dehydrated vegetables have been prepared to facilitate cooking, save preparation, and space in transit. The degree of destruction apparently depends upon the method used for dehydration. Sun drying results in destruction of far more vitamin C than artificial methods [862]. The material should be cooked or scalded before dehydration to inactivate the ascorbic acid oxidase ; sulphite is often added to retard the oxidation of the vitamin C. It has been recently shown that dehydration in an atmosphere of natural gas to exclude oxygen results in minimal losses of vitamin C [1080]. According to Moyer [905] considerable loss of vitamin C occurs in the commercial dehydration of vegetables, except in the case of cabbage. He states that destruction of vitamin C is almost complete in dehydrated potatoes. Davis and his co-workers [906] state, however, that only eleven to fifty-six per cent. of the vitamin C is lost in the dehydration of potatoes. Dehydrated vegetables lose further vitamin C on storage in the dry condition ; cabbage for example, stored at 87° C., loses fifty per cent. of its vitamin C in twelve weeks [907]. The rate of destruction of the vitamin C depends on the amount of moisture present, the degree of exposure to air and oxidising enzymes, pH and storage temperature. Dehydrated vegetables are best cooked by placing directly into boiling water and cooking for twenty minutes ; there is less loss than by soaking first in cold water and then heating up [910, 1057]. If dehydrated cabbage is first treated with cold water before cooking it loses sixty per cent. of its vitamin C ; when plunged directly into boiling water only eleven per cent. is destroyed [908]. Reconstituted dried cabbage contains about thirty-five per cent. of the original vitamin C in the fresh material, and reconstituted potato about twenty-five per cent. [1057].

The spray drying of liquids containing vitamin C does not cause

appreciable destruction of the latter [909]. Milk for example loses only twenty per cent. when spray dried [911]; roller dried milk loses about thirty per cent.; and evaporated milk about sixty per cent.

Vitamin C is destroyed in foodstuffs in a few hours by ultra-violet radiation. This is of little importance generally, but may be of some significance in the case of foodstuffs stored in glass containers exposed to light, *e.g.*, jam and milk. There is evidence that vitamin C in fruit syrup stored in glass bottles under normal conditions may be destroyed by sunlight [912]. This is important in the case of rose hip syrup and black currant purée which are issued as good sources of vitamin C to expectant mothers and young children. It would seem desirable to store these either in tins or amber-coloured bottles. This destruction only occurs in milk and processed foods. It may be different in natural foodstuffs exposed to solar radiation during ripening; the enzymes and pigments present probably afford protection [912]. Considerable losses of vitamin C may occur in milk left on the doorstep and exposed to daylight [1098]. It has been estimated that fifty per cent. or more may be destroyed in this way in an hour. The remedy for this is a carton or brown glass bottle [911].

Vitamin C Content of Foods. The vitamin C content of various foods before and after cooking and canning is given in the following table :—

Vitamin C Content of Foods

Foodstuff.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. (3½ oz.).	Remarks.
<i>Fruits.</i>	—	0.5–20	Av. 8.
Apple . . .	cooked	4	
	skin	6.1–15.5	
	jam and jelly	2	
	Bramley seedling	16	
	„ „ peel	88	
	Cox's orange pippin	1.6	
Apricot . . .	fresh	7	
	canned	5	
	dried	1–2	
	jam	2	
Avocado . . .		10	
Banana . . .	ripe	8.4–14.2	
Barberry . . .	raw	81	
Bilberry . . .	cooked	7	
Blackberries .	raw	7–20	
	cooked	8	20 when raw.
	jam	5	
	jelly	2	
Blueberry . . .	raw	4–75	Av. 10.
Cherry . . .	raw	2.8–7	
	cooked	8	
Cranberry . . .	juice	12	
Currant, black .	raw	108–419	Av. 150.
	cooked or canned	90	
	jam	50	

Vitamin C Content of Foods—continued

Foodstuff.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. ($3\frac{1}{2}$ oz.).	Remarks.
<i>Fruits—continued.</i>			
Currant, black .	juice	140	Ministry of Food.
	purée	70	
" red. .	raw	30-45	
	cooked	28	
	jam	6	
Custard apple .	raw	1.8-15	
Damson . .	cooked	8	
Elderberry . .	raw	8-10	
Fig . . .	fresh	2.2-8.72	
	dried	0	
Gooseberry . .	fresh	25-40	
	cooked	20	40 mg. when raw.
	jam	11	40 mg. when raw.
Grape . . .	raw	1-7.2	
Grapefruit . .	juice	24-45	
	oil gland layer	314	
	mesocarp	219	
	endocarp	76.6	
	canned	41	
	canned, sweetened	45-50	
	do. after 6 months	39-46	
	" " 12 "	34-41	
	marmalade	5.5	
Greengage . .	raw	5.0-6.5	
	cooked	2.7-5.7	5.0-6.5 mg. when raw.
	jam	2	
Guava . . .	—	75	
Haw . . .	raw	49-500	
Hip, rose . . .	fresh	67 up to 4,800	
	brought to boil	394	418 mg. when raw.
	boiled 5 min. with sugar.	238	"
	boiled 10 min. with sugar .	216	"
	jelly	100	
	syrup	175-200	Ministry of Health
Huckleberry . .	—	80	
Lemon. . . .	juice	30-78	Av. 45.
	pulp	14-16	
	peel	100-205	
	marmalade	10	42 mg. when raw.
Lime	pulp	20-60	
	juice	16.8-62.5	Av. 37.
Litchi	—	2; 20	
Loganberry . .	raw	20.4-48.4	
	boiled	22-26.7	38.8-48.4 mg. when raw.
	canned	31-35	" "
Mango	raw	25	
Medlar. . . .	raw	2.0	
Melon	raw	8	

Vitamin C Content of Foods—continued

Foodstuff.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. 3½ oz.).	Remarks.
<i>Fruits—continued.</i>			
Melon . . .	cantaloupe	30	
	water melon	1.0-7	
Mulberry . . .	raw	6.6-21	
Nectarine . . .	raw	8-24	
Olive . . .	raw	15	
Orange . . .	pulp	16-47	
	Brazil (pulp)	34-62	
	Jaffa "	33-54	
	navel "	52-98	
	juice	22-89	Av. 45.
	peel	75.8-210	
	canned	29.4	50 mg. when raw.
	marmalade	7-14	50 mg. when raw.
Papaya (pawpaw) .	raw	36-115	Av. 45.
	skin	116	
Peach . . .	raw	2-17	Av. 10.
	canned	3	
Pear . . .	raw	3-7	
	canned	1.5	
Peppers . . .	green	125-180	
	red, ripe	150	
Pineapple . . .	raw	25-60	Av. 20.
	canned	8	
Plum . . .	fresh	3-7	
	dried prune	1.0-2.0	
	boiled	2.2-2.9	4.6 mg. when raw.
	canned	2.2-2.5	" "
Pomegranate . . .	raw	6-15.6	
Pumpkin . . .	raw	5	
	cooked	2	
Quince . . .	raw	8	
Raspberry . . .	raw	19-37	Av. 25.
	canned	3.9-8	
	jam	8	
Rowan (mountain ash) . . .	—	35-50	
Strawberry . . .	raw	40-234	
	boiled	25-37	71.4 mg. when raw
	canned	21-35.7	" "
Tangerine . . .	pulp	10-36	
	juice	10-78	
Whortleberry . . .	—	5	
<i>Nuts.</i>			
Almond . . .		< 19.3	
Cashew . . .		1.79	
Coconut . . .		0.4-18.4	
Hazel . . .		2.74; 15	
Walnut . . .	unripe	400-3,000	
	chutney (home made)	98 per cent. of original	
	chutney (com- mercial)	40 per cent. of original	

Vitamin C Content of Foods—continued

Foodstuff.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. (3½ oz.).	Remarks.
<i>Vegetables.</i>			
Artichoke, globe .	raw	9	Av. 40.
	cooked	6	
„ Jerusalem		7	
Asparagus . .	whole	12-71.5	
	canned	15	
	cooked	30	
Bean, broad . .	raw	27.7-37	
	boiled	10	
	canned	14.7-17.6	
Bean, green, snap or string . .	raw	25-32	32 mg. when raw.
	cooked	15	
	dried	0	
Bean, soya . .	black, dried	40-46.66	
	green, dried	17.75	
Beetroot . .	root	15	
	cooked	5	
	canned	2	
	tops	50	
Broccoli . .	entire plant	65	
	boiled leaves	22	30 to 80 per cent. retained. Av. 70 per cent.
	dehydrated	6.0	
Cabbage . .	raw	62-105	
	cooked	29-51; 70	
	dehydrated (uncooked)	322.5	
	dehydrated (cooked)	42-62	
Carrot . .	raw	5-10	
	cooked	3-7	
	canned	2	
Cauliflower . .	raw	87-150	
	cooked	37-40	Av. 6-12.
	dehydrated (uncooked)	290	
	dehydrated (cooked)	17-60	
Celery . .	stalks	7-10	
Chard . .		35-42	
Chives . .		119	
Corn (sweet). .		10	
Cucumber . .		9-18	
Dandelion . .	leaf	100	
Endive . .	unblanched	15-24	
	blanched	4	170-295
Garlic . .		5-29	
Grass . .	fresh	68; 75.3	
Horseradish . .		105-136	
Kale . .	raw	126	
	boiled	23-40	
	dehydrated	170-295	

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Vitamin C Content in Foods—continued

Foodstuff.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. (3½ oz.).	Remarks.
<i>Vegetables—contd.</i>			
Kohlrabi . . .	raw	60-117	
Leek	raw	24	
	cooked	10-15	
Lettuce		12-15	
Lucerne (alfalfa) .	fresh	73-380	
Marrow	raw	2-5	
	cooked	2	
Mint		39	
Mustard and Cress		37	
	seeds	44	
Mushroom . . .		8	
Nasturtium . . .	leaves	200-465	
Nettle	leaves	50 ; 72	
Onion	bulb, raw	15-30	
	spring	25	
	cooked	6	
Parlsey	leaves	154-209	
Parsnip	raw	13-22	
	cooked	4-10	
Pea	fresh green	25	
	dried	0	
	boiled	14-16	
	quick freeze	12-23	
	canned	10	
Pepper	green	125	
	red	150	
Potato	tubers, raw	11-36	Av. 18.
	new	20-33	
	old	5-10	
	stored 180 days	10	18 mg. raw.
	baked	7	" "
	peeled and boiled	5-8	" "
	peeled, sliced and boiled	4	" "
	boiled whole	7-3	" "
	boiled whole cold	2-6	" "
	boiled and mashed	4-6	" "
	boiled and creamed	2	" "
	boiled and fried	2-1	" "
	dehydrated	16	60 per cent. retained.
	„ reconstituted	6	25 per cent. retained.
Pumpkin	cooked	2	
Radish	root	29-36	
Rhubarb	fresh	20-25	
	cooked	4	
	jam	2	
Shallot	bulb	7-6	
Spinach	leaves	50-80	
	boiled	15 ; 71	
	quick freeze	32	

Vitamin C Content of Foods—continued

Foodstuffs.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. (3½ oz.)	Remarks.
<i>Vegetables—contd.</i>			
Sprouts . . .	fresh	65-125	
	boiled	43-85	
	quick freeze	51	
Squash . . .	raw	29	
	cooked	7-17	
Swede . . .	root, raw	44	
	„ cooked	22	
Tomato . . .	raw	16-25	
	canned	14-21	
	late	5-8	
	canned after 6 mths.	7-18	
Turnip . . .	root	80	
	cooked	13-15	
	tops	153	
	boiled	18	35 mg. when fresh.
	„	17 per cent. destroyed.	
		28 per cent. diffused.	
Watercress . .		61-89	
<i>Dairy Products</i>			
Milk . . .	Cows' raw	0.7-2.96	
	„ colostrum	1.77	
	„ boiled	0.4	0.7 mg. raw.
	„ pasteurized	0.3-1.9	
	„ evaporated	0.4-2.76	
	„ skimmed, dried	1.58-6.27	
	„ whole dried, by roller process	6.9-9.7	
	Goats' raw	0.5-2.0	
	„ boiled	0.4	0.9 mg. fresh.
	Human	1.2-10.8 (4-8 average)	
	„	8-10	well-fed mothers.
	„	1.2-4.0	poorly fed mothers.
	„	7.0	colostrum.
Cheese. . .		nil	
Eggs, hen's . .		nil	
Eggs, duck's. .		0.3-1.3	
<i>Fish.</i>			
Carp . . .	liver	4.5-11.8	
	muscle	0.5-1.88	
	roe	20-24	
		5.7; 8.3	
Clam . . .		26.7	
Cod . . .	liver	120-160	
	roe	27	
Crab . . .	liver	3.5	
	muscle		
Eel . . .	liver	9.8-11	
	muscle	1.4	

Vitamin C Content of Foods—continued

Foodstuff.	Description.	Vitamin C in mg. per 100 gm. or 100 c.c. (8½ oz.).	Remarks.
<i>Fish—continued.</i>			
Haddock . . .	roe	10	
Herring . . .	roe	20	
Lobster . . .	liver	24	
	muscle	5	
Mackerel . . .	roe	40	
Mussel . . .	liver	30	
	muscle	8	
Oyster . . .	liver	12	
	muscle	8	
Salmon . . .	whole fish	89-215	
	chilled	9	
	canned	0	
	roe	14	
Scallop . . .	liver	11.5	
	muscle	3	
<i>Meat and Poultry.</i>			
Calf . . .	liver	30-50	
	muscle	7.8	
Duck . . .	liver	13 ; 68	
	muscle	7.8	
	heart	24.2	
Fowl . . .	muscle	1.5-6.0	
	liver	28-43	
	brain	11.4-26	
	heart	3.8 ; 4.6	
	kidney	10.8	
	liver	35	
	muscle	1.6 ; 2.2	
Ox . . .	liver	27-40	
	muscle	1.7	
Pig . . .	brain	25	
	kidney	14	
	liver	11-41	
	muscle	1.9	
Rabbit . . .	muscle	0.42-3.4	
Sheep . . .	brain	15.4	
	heart	6.2	
	liver	25-50	
	kidney	10.9	
	muscle	2.5	
<i>Miscellaneous</i>			
Beer . . .		nil	
Corn . . .		2.1	
Cider . . .		trace	
Coffee . . .	freshly ground	56-61	
Honey . . .		0-20	
Rice . . .		2.4	
Tea . . .	dried	nil	
	fresh leaves	120	
Wheat . . .		2.6	
Yeast . . .	bakers'	1.6	

PHYSIOLOGY AND FUNCTIONS OF VITAMIN C

Vitamin C is synthesized by all the higher plants, probably by simple organisms such as moulds and bacteria, and by many animals except the guinea-pig and the primates. The rat and the cow are able to synthesize vitamin C [865, 915]. It is formed during the germination of seeds.

Collagen Formation. One of the functions of vitamin C that has been fairly well established is its rôle in the formation of intercellular substance in the animal organism [89, 40]. Wolbach and his associates [41-44] in a series of papers from 1926 to 1937 showed that reticulum and collagen are not formed in the scorbutic animal. Normally in the intercellular substance fibroblasts lie in an amorphous ground substance within which fibrils of a reticulum are found as wavy bands of collagen. The fibrils are cemented together by a translucent matrix. In the scorbutic animal the ground substance and fibroblasts are present but no collagen is formed. After the administration of vitamin C, bundles of collagenous material are formed within twenty-four hours. It is also claimed that vitamin C accelerates the deposition of intercellular material when added *in vitro* to a tissue culture containing living fibroblasts [45]. The rôle of vitamin C as a prerequisite for the formation of intercellular material in the human subject has been established without doubt by the work of Hunt [77] and Crandon [68] (pp. 468, 582). The manner in which vitamin C acts in the formation of intercellular material is unknown.

This function of vitamin C offers an explanation of the observations of the seafarers of previous generations, who described the opening of healed wounds, the breaking down of the callus of old united fractures, and the weakening of ligaments in scorbutic sailors.

Structure of Teeth. The intercellular substances regulated by vitamin C also include those of dentine, cartilage and the matrices of bone. As early as 1919 Zilva and Wells [46] showed that radical changes occur in the teeth of scorbutic animals. We are indebted to Höjer [47] for a detailed description of the effects of vitamin C deficiency on the teeth. He observed the following changes in the perpetually growing teeth of guinea-pigs:—

(1) Changes occur in the odontoblasts, which become shorter and spindle-shaped, and indistinguishable from the tissue cells in the pulp of the tooth. If the deficiency is partial and prolonged for several months the odontoblasts produce a substance resembling bone, which gradually fills the pulp canal.

(2) The widening of Tome's canals in the dentine, which is resorbed and porotic.

(3) Instead of new dentine, spongy, porous, bonelike material (osteodentine) is formed.

(4) Pulp tissue, pulp cells and the newly formed bone become atrophied and resorbed, and small cysts and foci of calcification are seen.

(5) These lesions commence in the crown and extend towards the root. Alveolar resorption in the jaw occurs and extends to the outer edges of the mandible in animals kept on a diet deficient in vitamin C for about four weeks [60]. The earliest signs of scurvy in the guinea-pig are found in the teeth.

Höjer's work has been criticized by Wolbach and Howe [41], who state that Höjer's conclusions were based on incomplete scurvy. In complete scurvy they describe a shrunken pulp completely detached from the dentine and apparently floating in a liquid material; contrary to the finding of Höjer no bone was observed.

Fish and Harris [48], who have detected defects in the enamel and cement as well as in the dentine of scorbutic guinea-pigs, consider that vitamin C is essential for the functional activity of formative cells such as odontoblasts, osteoblasts, ameloblasts, etc. Impaired nutrition of the ameloblasts may lead to serious defects in enamel formation, and since these changes are irreversible the tooth is in danger of decay. Boyle [49], however, states that although hypoplastic areas of enamel are seen in scorbutic guinea-pigs, they are not present in infants suffering from scurvy.

Care is needed in applying the results of guinea-pig experiments to man. In the guinea-pig the teeth go on growing, whereas in man the growth of the teeth ceases once they are fully formed.

There is no clinical evidence that dental decay in man is due to vitamin C deficiency [50, 51, 916]. Grandison [916] treated children aged four to fourteen with vitamin C and gave them no local treatment; a control group were given local treatment but no vitamin C. He found the incidence of dental caries greater in the vitamin C group than in the controls. This does not, of course, exclude the view that vitamin C is essential for tooth structure during the formative period.

Bleeding, soreness of the gums and loosening of the teeth in human beings—so-called periodontal disease—may possibly be associated with vitamin C deficiency, and are stated to improve with intensive administration of the vitamin [52-57]. The subject is further discussed on pp. 538, 566.

Bone Formation and Repair. Vitamin C is also essential for the formation of bone and cartilage (see Fig. 112). The gross and histological changes seen in the bones of scorbutic animals are similar to those seen in other structures. The lesions are commonest at the costochondral junctions, the distal end of the femur, the proximal end of the tibia, femur and wrist. In the affected bones bone formation ceases and the existing osseous shell becomes rarefied, with an increased tendency to fracture on slight trauma, particularly at the epidiaphysial junctions in growing bones, and false motion at the costochondral junctions. These are the results of failure to form intercellular material. In young bone the osteoblasts lose their characteristic shape and migrate from the trabeculae to the diaphysis. Formation of cartilage and bone matrices ceases. The osteoblasts become surrounded by liquid and give rise to an apparent region of oedematous connective tissue at the ends of the diaphysis, the *Gerüstmark* (framework marrow) of German authors. Microscopically rarefaction of the cortex is observed, bone ceases to grow, and the normal junction is replaced by a zone of connective tissue poor in collagen and in which are embedded fragments of calcified cartilaginous matrix, devoid of osteoid tissue. Controlled experiments show that the connective tissue cells of the marrow are osteoblasts that have migrated and reverted to fibroblasts. Vitamin C appears to be necessary for the proper functioning of the osteoblasts, which in its

absence revert to their prototype and attempt to form a fibrous union between epiphysis and diaphysis.

Owing to the continued proliferation of the osteoblasts in the periosteum of the bones of scorbutic animals, stripping of the periosteum from the bone cortex occurs. Kodicek and Murray [100] have observed marked overgrowth of subperiosteal bone and porosis in animals suffering from prolonged vitamin C deficiency. Lack of intercellular material in the neighbouring blood vessels leads to subperiosteal hæmorrhages, which strip the periosteum off the bone and are characteristic of the scorbutic state (Fig. 149). The response to treatment with vitamin C is dramatic. Within twenty-four hours new intercellular substance is formed, the fibroblasts are surrounded by a thin shell of osteoid material, trabeculae are formed, proliferation of osteoblasts in the periosteum ceases, and hæmorrhage from the fragile capillaries stops. Capillary formation, which is essential in growing tissue, is resumed.

Mouriquand [61-63] has described the bony defects resulting from acute and chronic vitamin C deficiency. In acute deficiency softening and rarefaction of the bone occurs, particularly in the region of the femoral or tibial epiphyses. In chronic deficiency he describes a syndrome resembling chronic osteo-arthritis, with osteophytic outgrowths, pseudo-ankylosis, decalcification of the epiphyses and diaphysis, and periosteal thickening. Complete decalcification and disappearance of the neck and head of the femur have also been observed. Periostitis and an ankylosing arthropathy associated with infantile scurvy have been described [64, 65]. These observations on the relationship between vitamin C and bone changes in scurvy may require some modification in view of the observation of Follis [917] that there is a complete absence of bone disorganization in the limbs of scorbutic guinea-pigs if they are immobilized in plaster.

Until recently it was not known that vitamin C is essential for the production of bone salts. Wolbach and Bessey [918], writing in 1942, stated that vitamin C plays no part in the process of calcification. Bourne [919] has now shown that the deposition of bone salt in both normal and regenerating bone is retarded in scorbutic animals, but not in the bone of animals receiving adequate vitamin C. He suggests that the function of vitamin C in bone formation is to facilitate the production, not only of bone matrix, but of bone matrix impregnated with phosphatase, which is essential for calcification. A deficiency of vitamin C lowers the alkaline phosphatase of both bone and blood [1069].

Vitamin C is essential for the formation of callus in the union of fractured bones. Fractures heal badly in a subject, human or animal, deficient in vitamin C. Wolbach and Howe [41, 42] have demonstrated the complete failure of natural callus formation in experimental scurvy and the resorption of old callus and the refracture of united fragments of bone has been described in scurvy since early times. It has also been shown that the excretion of vitamin C falls rapidly in an animal with multiple experimental fractures [56] and that the degree of healing in bone is proportional to the amount of vitamin C in the diet. Bourne [920] has shown that the optimum formation of bony trabeculae in the injured femora of guinea-pigs is brought about by the administration of 2 mg. of

vitamin C daily (probably equivalent to 40 mg. in a human being) and that anything less than 1 mg. seriously retards the formation of bony trabeculae. Bourne [921] has also shown that neither vitamin C nor calcium administered parenterally accelerate bone healing in rats, but that calcium ascorbate injected subcutaneously does.

A definite relationship between periosteal activity in the healing of injured bone and the amount of vitamin C in the diet has been demonstrated by Bourne [919, 922]. In an animal receiving no vitamin C there is no trace of a reaction by the cambial layer of the periosteum at the end of a week. The activity of both the periosteum and endosteum in healing bone is impaired by a deficiency of vitamin C. Bourne's observations suggest that it is desirable to maintain the vitamin C at an optimal level in patients with fractures.

In the scorbutic animal defective capillary formation in the region of a fracture can be demonstrated; after the administration of vitamin C capillary proliferation and active hyperaemia occur [59].

Italian workers have shown that if vitamin C is given to rabbits with experimentally produced fractures, rapid formation of callus results and a speedy return of function. In controls not receiving vitamin C complete union, which was confirmed radiologically, took twice as long as in the animals treated with vitamin C [66]. Excessively large doses of the vitamin exert a deleterious effect during the first few days.

Bourne [921] has been unable to show that additional vitamin C has any effect in accelerating the regeneration of bone in rats, which synthesize their own vitamin C.

Wound Repair. Since vitamin C is essential for the formation of intercellular material it is necessary for wound repair. Lauber and Rosenfeld [69] demonstrated its presence in appreciable quantities in young granulation tissue and adjoining skin areas by the cytological staining technique developed by Bourne [70], Gough [387], Giroud and Leblond [71]. This depends upon the fact that vitamin C is the only substance occurring in cells that reduces silver nitrate to metallic silver in the presence of acetic acid (see Figs. 136 to 142). This histo-chemical detection of vitamin C is apparently specific for normal tissue, but according to Wolff-Heidegger [928] it is unreliable when normal metabolism is disturbed, *e.g.*, after adrenalectomy or castration. Lanman and Ingalls [72], Taffel and Harvey [73], Bourne [925] and others [85] have shown that the tensile strength of healing wounds in guinea-pigs suffering from a partial deficiency of vitamin C is considerably less than in normal animals (Figs. 120-128). The former investigators showed that wounds from operative incisions in guinea-pigs partially deprived of vitamin C ruptured at a pressure of only a third of that required to rupture wounds of normal animals. The scar tissue was also distinctly abnormal; there was a marked decrease in the intercellular material and a disorganized arrangement of the fibroblasts. These observations have been confirmed by Hartzell and Stone [924] and by Bourne [925], who improved the technique used for investigating the tensile strength of wounds. Bourne [925] found a relationship between the total blood vitamin C and the tensile strength of the wound. "Saturation" with the vitamin is not essential for optimal healing; in the guinea-

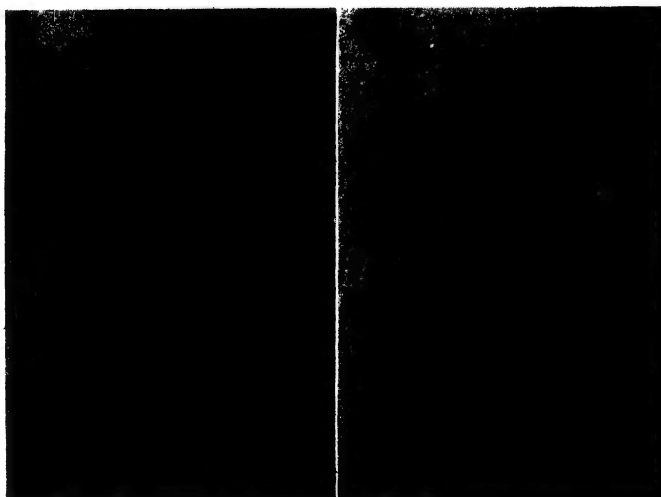
VITAMIN C AND WOUND HEALING



FIG. 112. Growing point of cartilage in a chick embryo, showing cartilage cells laden with vitamin C granules. Silver stain ($\times 800$).



FIG. 113. Vitamin C and Wound Healing. Section of wound in skin of cat after seven days. In the scar tissue are numerous histiocyte-like cells containing granules of vitamin C. Silver stain ($\times 300$).



Control.

Subcorbutic.

FIGS. 114 and 115. Vitamin C and Wound Healing. Abdominal incisions of guinea-pigs twenty-one days after operation (life size). Both healed by first intention. *Left*, control animal receiving adequate vitamin C; *right*, subcorbutic animal. In the control the scar is practically invisible. In the subcorbutic animal it is puckerred, stretched, sunken and shows a mauve discoloration.

VITAMIN C AND WOUND HEALING



FIG. 116. Control.

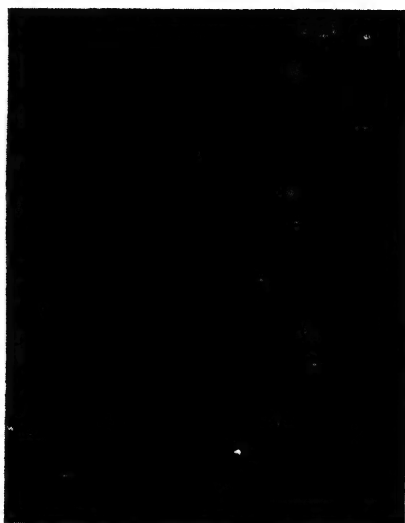


FIG. 117. Subscorbutic.



FIG. 118. Control.



FIG. 119. Subscorbutic.

FIGS. 116 to 119. Vitamin C and Wound Healing. Sections from the abdominal scars of guinea-pigs killed twenty-one days after operation ($\times 800$). Figs. 116 and 118 are sections from a control animal; Figs. 117 and 119 from a subscorbutic animal. Fig. 116 shows fibrocytes and Fig. 117 fibroblasts, stained with hæmatoxylin and van Gieson stain. There is no van Gieson staining intercellular substance in the scar of the subscorbutic animal. Fig. 118, stained with silver, shows black silver-stained reticulum fibres only around the blood vessels. The rest of the ground or intercellular substance is mature collagen and appears translucent yellow in section. In contrast in Fig. 119, from the subscorbutic animal, the whole of the intercellular substance consists of coarse irregular bands of pre-collagen, which stains densely black with silver.

pig a daily intake of 2 mg. is adequate, and any increase beyond this has no significant effect on the tensile strength of the wound (Figs. 120-128). Hartzell and Stone [924] found that if scorbutic animals were operated on wound healing was satisfactory if they were given sufficient vitamin C after the operation. The effect of vitamin C on wound healing is apparently specific as citrin or vitamin P has no effect on the process in either normal or vitamin C deficient animals [924]. Jones and his colleagues [926] have further shown that the tensile strength of the scar of an operation wound in guinea-pigs is proportional to its vitamin C content. Hunt [77] also observed that in the wounds of guinea-pigs deficient in vitamin C the scars were puckered, stretched, sunken, loose, irregular and almost avascular, and that the removal of catgut ligatures, either by phagocytosis or by extrusion, was delayed (see Figs. 114, 115, 155). Others have demonstrated

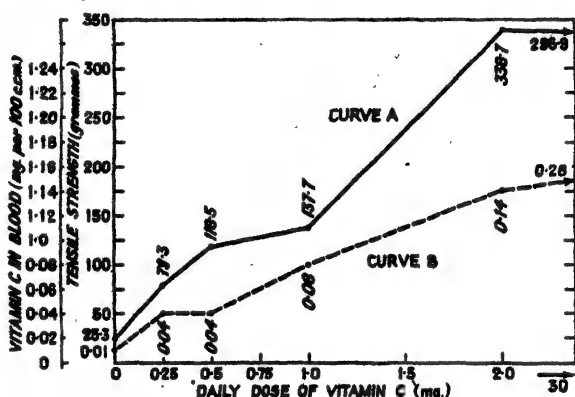


FIG. 120. Tensile Strength of Wounds and Vitamin C Intake. Curve A, tensile strength of wounds in fifty-two guinea-pigs on varying intakes of vitamin C; the tensile strength of wounds increases with increasing vitamin C intake, up to an optimum of 2 mg. Curve B is the total blood vitamin C in fourteen guinea-pigs on varying doses of vitamin C. The blood vitamin C rises with increased intake of vitamin C. A maximum is reached when the intake is 2 mg. daily.

that scarified duodenal mucosa failed to heal in guinea-pigs deficient in vitamin C, but healed promptly in the control animals [74]. It has also been observed that on a high intake of vitamin C the tissues of the healing area around a wound contain more vitamin C than those from normal controls; if the organism is on a scorbutic diet there is no increase in vitamin C in the healing area [851]. These findings receive support from the clinical observations of a number of workers, notably those of Wolfer and Hoebel [75], Archer and Graham [76], Crandon [68], Hunt [77] and Bartlett [852].

The clinical evidence of the necessity of vitamin C for wound healing was somewhat open to question until the work of Crandon [68], since the cases reported were poorly controlled with respect to other variable factors important in the healing of wounds (serum protein, surgical technique, infection, other avitaminoses) and tissues were not subjected to biopsy. Crandon placed himself on a vitamin C-free diet, supplemented

VITAMIN C AND WOUND HEALING



FIG. 121. Unorganized blood clot in wound of a guinea-pig receiving no vitamin C. Masson trichrome stain ($\times 100$). Section made after a week's healing. Tensile strength of wound 46 grams.



FIG. 122. Section of wound of guinea-pig receiving 0.25 mg. vitamin C daily. Hæmatoxylin and van Gieson stain ($\times 50$). Little scar tissue is present. Although the surface of the wound is covered by epithelium, there is a large empty space beneath the epithelial layer. Tensile strength of scar 84 grams.



FIG. 123. Section of wound of guinea-pig receiving 0.5 mg. vitamin C daily. Hæmatoxylin and van Gieson stain ($\times 50$). There is a well-marked difference between the normal connective tissue and scar tissue. This is due to the small amount of fibres and large numbers of cells in the scar. The epithelium has covered the scar completely. Tensile strength of scar 62 grams.



FIG. 124. Reticular preparation made from wound shown in Fig. 123 ($\times 120$). On the left is normal connective tissue, on the right are numerous reticular fibres and cells in the scar.

VITAMIN C AND WOUND HEALING



FIG. 125. Section of wound of guinea-pig

scar contained about the same amount of fibrous tissue as the scar in the guinea-pigs receiving 2 mg. of vitamin C (Figs. 127, 128), but more of the fibres were reticular. Tensile strength of scar 158 grams.

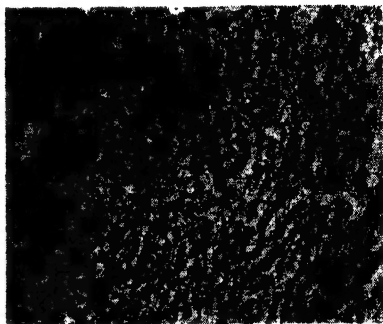


FIG. 126. Reticulum preparation of wound, section of which is shown in Fig. 125. Numerous fine reticular fibres, but few cells, are present.



FIG. 127. Section of wound of guinea-pig receiving 2 mg. of vitamin C daily. Hæmatoxylin and van Gieson stain ($\times 50$). There is abundant collagen in the scar. Tensile strength of scar 295 grams.



FIG. 128. Reticulum preparation of wound section of which is shown in Fig. 127 ($\times 50$). Few reticular fibres are present and the intensity of staining of scar tissue is similar to that of normal connective tissue.

by all the other known vitamins, for a period of six months. At the end of three months a good sized wound was made in the right mid-back. Ten days later biopsy of this wound showed good healing, compared with a normal control, and histological examination showed ample intercellular substance and capillary formation (Fig. 129). After just over six months, when the plasma vitamin C had been zero for five months, and three weeks after the signs of frank scurvy, a similar wound to the first was made. Healing appeared to progress, but on the tenth post-operative day a biopsy of the wound was made and it was found that beneath the skin, which appeared well healed, there was no healing of the wound, which was filled with unorganized blood clot (Fig. 180). So little healing had occurred that it was found necessary to insert a small rubber drain. Sections of the wound showed a lack of intercellular substance and capillary formation, confirming the work of Wolbach on the wounds of scorbutic guinea-pigs (Fig. 182). Immediately after the biopsy the patient received an intramuscular injection of 1,000 mg. of vitamin C. Ten days later another biopsy specimen was excised from the wound (Fig. 181). There was good healing, the sections showing ample intercellular substance. This experiment proves beyond doubt that wounds fail to heal in cases of frank scurvy, but at just what point between three and six months on a scorbutic diet delayed healing begins we do not know. The fact must not be lost sight of, however, that the healing of a wound is dependent on many factors besides vitamin C.

Farmer [1078] records similar observations made in 1944 on volunteers kept on a scorbutic diet (0 to 10 mg. vitamin C daily) for many months. An incision was made in the thigh of some of the subjects and biopsy sections made at intervals five to fourteen days after healing. It was observed that the wounds were susceptible to infection—two of the volunteers had to be hospitalized on account of this—and that there was diminished tensile strength along the suture line and deficient formation of reticulum cells and collagen.

Hunt [77] kept a young healthy male on a vitamin C-free diet for three months, and like Crandon found that up to this period wounds healed normally, and were not different macroscopically or microscopically from wounds made in the same subject shortly after the administration of a large dose of vitamin C (Figs. 152 and 153). In a study of twenty-eight surgical cases that came to post mortem it was shown that those patients who had been most deficient in vitamin C showed the poorest collagen production. Wounds most commonly break down on the tenth day, and it is possible that by saturating surgical patients routinely with vitamin C, this important cause of wound disruption may be eliminated.

As a result of inadequate collagen formation in the subject deficient in vitamin C, the whole architecture of the healing wound is disturbed. The proliferation of fibroblasts occurs but there is no intercellular material (Fig. 117). The cells remain immature and blood vessels do not easily penetrate the poorly developed granulation tissue. Leakage of blood from the fragile capillaries forms hæmatomata, which are not organized or absorbed, and the scar is split by them or by extravasations of blood (Figs. 121 and 180). Phagocytosis is delayed and the surrounding

WOUND HEALING IN EXPERIMENTAL HUMAN SCURVY



FIG. 129. Section from wound of a human subject on a scorbutic diet for three months. An experimental wound was made and sutured and a biopsy specimen obtained eleven days later. Normal healing has occurred (see text, p. 469).



FIG. 130. Section from wound of a human subject on a scorbutic diet for six months, showing absence of healing. The wound appeared to heal by first intention. Ten days later a biopsy specimen was taken. As soon as the skin was divided it was found that the tissues under the skin had not healed at all and that the wound contained firm dry blood clot. This is shown in Fig. 130 by the large empty space beneath the epidermis (see text, p. 471).



FIG. 131. Same case as Fig. 130 after ten days' treatment with 1 gram of vitamin C daily. Another incision was then made across the same wound that had formerly failed to heal. A section of this shown in Fig. 131 shows wound healing has occurred.

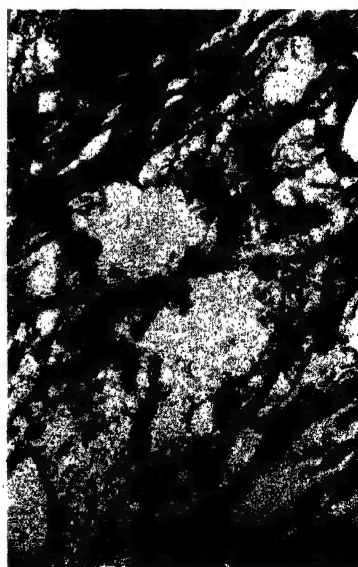


FIG. 132. Section from wound of human subject on a scorbutic diet for six months. Note formation of granulation tissue without intercellular substance.

supporting structures are not incorporated in the scar tissue of the wound.

Hartzell and his colleagues [845] examined the vitamin C level in two hundred surgical patients. Many of them showed low values when they came to operation, and many showed a drop in their vitamin C level after. It was also observed that those whose wounds subsequently disrupted or everted had low blood vitamin C values.

Bartlett and his colleagues [852] have shown that after surgical operations the tensile strength of wounds is decreased if the plasma vitamin C level falls below 0.2 mg. per 100 c.c. They also state that in spite of a low plasma vitamin C at the time of operation, normal wound healing may occur if adequate vitamin C is given post-operatively.

A discussion on vitamin C and wound healing from the clinical aspect will be found on p. 581.

It has been shown that skin grafts do not take well in scorbutic animals compared with controls receiving a diet adequate in vitamin C. If the diet is sufficiently scorbutic the grafts do not take at all, but become mummified [927]. This would suggest that it is wise to maintain an adequate vitamin C intake in patients receiving skin grafts.

Abscesses do not heal well in scorbutic animals. The appearance and number of macrophages is delayed, bacteria readily escape from the abscess into the surrounding tissues, and the necrotic centres in the abscess are not walled off [928].

Hines and his co-workers [1059] have shown that vitamin C is essential for the regeneration of damaged nerve. The tibial nerve of guinea-pigs was crushed and the rate of regeneration studied on diets containing graded amounts of vitamin C. Regeneration was impaired when the vitamin C intake fell below an optimal level of 2.5 mg. daily. It does not follow that vitamin C has any specific action on nerve tissue, as any deficiency in collagenous intercellular material in the tissues supporting a nerve would retard its regeneration.

Capillary Resistance. The hæmorrhagic manifestations of scurvy have been stated to be due to abnormal capillary fragility. Morphological changes have not been detected in the capillaries, and, although it has been suggested, it has not been proved that this increased fragility is due to a defect in the cement substance binding the capillary endothelium or in the pericapillary connective tissue and collagen sheath. Actually the minute structure of the capillary wall is not well known.

Crandon [68] noted that even after five months on a scorbutic diet, when petechiæ were beginning to appear over the legs, the application of a blood pressure cuff blown up to 100 mm. of mercury brought out no more petechiæ than in normal controls. In experiments on scorbutic guinea-pigs Dalldorf [79] found that the capillary resistance did not decrease constantly; actually it increased to normal values during the second week. Wachholder [80] and Rapaport [81] observed similar fluctuations in human cases. As a result of examining a hundred and fifty children the latter investigator was unable to correlate abnormal capillary fragility and vitamin C deficiency, as determined by blood tests.

• Göthlin [82, 83], Bell [84] and Dalldorf [85] have sought to establish a

correlation between capillary fragility and the degree of vitamin C nutrition. It cannot be admitted that a clear relationship between the two has been demonstrated. In fact, there is a growing body of evidence negating any relationship between them [68, 847, 850, 852, 527-530, 558, 869, 877]. Scarborough and Gilchrist [929] were unable to correlate increased capillary fragility and vitamin C deficiency as measured by plasma vitamin C levels. They actually found that in many cases the capillary fragility increased with a rising blood vitamin C.

Many other factors influence capillary fragility, such as menstruation, severe infections, malignant hypertension, hæmorrhagic states, diabetes and old age [980]. Recent work would suggest that vitamin P plays a part in the control of capillary fragility, since naturally occurring substances containing both vitamins P and C produce an increase in the capillary resistance of some patients, even when vitamin C has failed to produce any effect (p. 861). Scarborough [86] suggests that two forms of subcutaneous bleeding may occur as a result of avitaminosis. One is due to vitamin C deficiency and is characterized by ecchymoses involving considerable areas of subcutaneous tissue and muscle; it is not controlled by vitamin P. The other, which results from vitamin P deficiency, manifests itself in the form of spontaneous petechial hæmorrhages, particularly in areas exposed to pressure (*e.g.*, of tight clothes); it does not respond to the administration of vitamin C. The subject of vitamin C and capillary fragility is further discussed on pp. 505, 589.

Vitamin C and the Hæmatopoietic System. Witts [981] believes that for the maturation of the red cell from normoblast to erythrocyte iron, copper, thyroxine and vitamin C are necessary. In the absence of vitamin C the bone marrow becomes hyperplastic and normoblastic. Anæmia is frequently associated with scurvy. In 1930 Mettier, Minot and Townsend [88] noted that a reticulocyte response occurred in patients suffering from anæmia associated with scurvy when they were given orange juice. They suggested that the anæmia of scurvy was due to the prolonged effect of vitamin C deficiency altering the functions of the bone marrow. Atrophy of the bone marrow in scorbutic guinea-pigs has been described; microscopically the marrow appears to be filled with areas of an amorphous material resembling amyloid [89]. Evidence has been brought forward to show that the anæmia of the scorbutic guinea-pig is due to retardation of the process of maturation of the erythrocytes in the bone marrow [90]. Stimulation of the bone marrow of scorbutic guinea-pigs is said to follow the injection of vitamin C [108]. On the other hand, an increase in the reticulocytes has been described in some cases of human scurvy and also in guinea-pigs in an advanced stage of untreated scurvy [91, 94].

Parsons and Smallwood [982] believe that the anæmia often found in scurvy is due to a general slowing down of the whole process of erythropoiesis, which may be so marked as to result in marrow degeneration and aplasia, the resulting anæmia being usually orthochromic and normocytic. In chronic cases associated with hæmorrhage into the tissues and from mucous membranes a post-hæmorrhagic blood picture may be superimposed and the blood picture may be hypochromic and in extreme cases may be microcytic.

Although anæmia is quite often met with in human scurvy, its occurrence is inconstant. Thus Crandon [68] existed on a scorbutic diet for six months and in spite of a mild chronic loss of blood resulting from various blood determinations, and a vitamin C deficiency of 6,000 mg., he did not develop anæmia. The hæmoglobin showed a slight fall during the third month on the scorbutic diet, but rose to normal levels after the administration of 0.5 gm. of ferrous sulphate daily. Certainly in the scorbutic infant anæmia is inconstant and is not an essential symptom of vitamin C deficiency [92, 98]. Mild vitamin C deficiency is not associated with anæmia [870]. When anæmia accompanies vitamin deficiency it may well be that the anæmia is due to a generally deficient diet in which substances other than vitamin C, particularly iron, have been lacking.

Clinically many cases of nutritional anæmia have been described which failed to respond to iron, but did to vitamin C. Parsons [95, 96] has observed anæmias of the normocytic and macrocytic types which were cured by the administration of vitamin C, without any other form of therapy. He believes that vitamin C acts on the cells of the bone marrow throughout the whole range of maturation of the red cell, from the reticulo-endothelial cell to the adult erythrocyte, and not over a small portion of its maturation. Dunlop and Scarborough [94] also showed that a rise in the red cell count, a reticulocytosis and a rise in the hæmoglobin percentage resulted when vitamin C was given to a scorbutic patient, although the iron content of the diet was not increased. Similar observations have been reported in scorbutic infants [97]. Jennings and Glazebrook [98] have described cases of scurvy with associated anæmia that responded to vitamin C alone. One case with a megalocytic anæmia showed reticulocytosis, polychromasia and a hyperplastic bone marrow; another showed a normocytic anæmia. The latter failed to respond to either liver or iron, but was cured solely as a result of vitamin C therapy. Aron [99] also states that anæmia can be produced in rats by depriving them of vitamin C. This anæmia is not cured by iron, but only by administering vitamin C. Lozner [110] has reported cases of vitamin C deficiency with anæmia that did not respond to the administration of vitamin C. Satisfactory hæmoglobin regeneration occurred without vitamin C after giving the vitamin B complex.

It is possible that the absorption and utilization of iron are defective when the reserves of vitamin C in the body are depleted. Liu [684] administered 50 mg. of vitamin C a day for four weeks to forty-four boys suffering from anæmia and vitamin C deficiency. The blood vitamin C level rose from an average of 0.28 mg. to 0.91 mg. per 100 c.c., but the hæmatocrit or hæmoglobin values and the reticulocyte count were unaffected. When, however, the boys were given 1.5 gm. of ferrous carbonate a day there was a significant rise in hæmatocrit, erythrocyte and hæmoglobin values. This occurred in cases where the blood vitamin C levels were very low. There is a close correlation in human subjects between the amount of vitamin C in the blood and the amount of hæmoglobin [988] and of iron, the level of which can be raised by giving vitamin C [984]. It has been suggested that vitamin C may play a rôle in the absorption of iron.

The studies of Barron [101] suggest that vitamin C may play an important rôle in maintaining the hæmoglobin of the blood at a normal physiological level. He found that vitamin C reduced the red blood count to normal levels in animals in which polycythæmia had been artificially induced by administering cobalt salts. This experimental polycythæmia is probably caused by the cobalt inhibiting the respiratory function of immature red blood cells which are rapidly replaced in the bone marrow. Vitamin C, however, has no effect on the polycythæmia produced by continued exposure to low atmospheres [725]. Deeny [726] investigated the effect of vitamin C in two cases of human polycythæmia. The vitamin alone had little effect on the condition, but in conjunction with sodium bicarbonate it caused a marked fall in the red blood count to normal levels. Kandel and Le Roy [727] failed to note any improvement in patients with polycythæmia treated with vitamin C.

In vitro there is some evidence that there is a coupled oxidation of vitamin C and hæmoglobin, the reaction leading to the formation of bile pigments from the hæmoglobin [988]. If these reactions occur *in vivo* then vitamin C plays an important rôle in hæmoglobin catabolism.

Infection and Immunity [118-115]. A considerable amount of indirect evidence suggests a relationship between vitamin C and infection.

Increased Requirements of Vitamin C in Infection. Well controlled animal and human studies have shown that in infections there is a lowering in the excretion of the vitamin and a fall in the blood level of vitamin C [119-129]. Thus Faulkner and Taylor [125] found that the average vitamin C content of the blood serum of healthy persons is 1.31 mg. per 100 c.c., whereas in patients suffering from infectious diseases it is as a rule fifty per cent. lower, that is about 0.65 mg. per 100 c.c. Harris [119-128] and his collaborators, who have carried out large scale surveys on the excretion of vitamin C in a variety of infective states in nearly 500 cases, have come to the following conclusions: (a) In the active stage of infections the excretion of vitamin C is very low; a lowered excretion is a reflection of increased utilization and hence increased requirements. The severity and virulence of the infection is proportional to the lowered excretion. (b) The vitamin C excretion in cured cases (except rheumatism) is quite normal. (c) The excretion of vitamin C in semi-active cases is intermediate.

Examination of the vitamin C reserves of the body (blood, urine, saliva, cerebrospinal fluid) in pulmonary tuberculosis, pneumonia, diphtheria, and febrile diseases generally reveals that they are low in comparison with normal standards [180-185]. A comparison of the vitamin C content of the organs of experimental animals, suffering from infections such as diphtheria and tuberculosis, with those of healthy controls shows that the stores of vitamin C are depleted in the infected animals [186-188].

One must distinguish between increased utilization of vitamin C due to elevated body temperature and increased utilization due to an infectious process *per se*. Abt and his colleagues [152] concluded that pyrexia *per se* does not significantly lower the vitamin C level of the blood nor increase the utilization of vitamin C.

Vitamin C and Resistance to Infection. Investigations have been made

to correlate the intake of vitamin C in animals and human beings with their immunity and resistance to infection [116-118, 140-151].

Animal experiments have shown that vitamin C has a stimulating effect on the production of antibodies [149]. It is claimed that it increases the agglutinin, hæmolyisin and precipitin titres and the opsonic index of experimental animals treated with vaccines or toxoids. Juszat [149] states that 100 mg. of vitamin C added to an immunizing dose of horse protein causes a five to seven-fold increase in specific precipitin production in rabbits; this has been confirmed by Madison and Manwaring [158]. Others by using optimum amounts of vitamin C (50 mg.) brought about a ten to thirty-fold increase in the precipitin titre [148]. Cameron [140] found that the serum of guinea-pigs previously injected with diphtheria toxoid and receiving 8.6 mg. of vitamin C a day contained more antitoxin than the serum of animals similarly treated, but only receiving a quarter of this amount of vitamin C a day.

The effect of vitamin C on the tuberculin reaction in tuberculous animals has been studied by Birkhaug [150], who infected guinea-pigs with tubercle bacilli and administered 10 mg. of vitamin C a day to the animals; controls received an adequate diet, but no extra vitamin C. He concluded that the daily administration of vitamin C significantly inhibits the tuberculin reaction in tuberculous guinea-pigs, and that the degree of inhibition of the tuberculin reaction is definitely correlated with the urinary excretion and the adrenal content of vitamin C in the tuberculous animal. Birkhaug also found that vitamin C had a curative effect in that it caused a significant increase in body weight and a reduction in the number and extent of tuberculous lesions. Microscopic examination revealed fewer caseonecrotic lesions, more collagen tissue and fewer tubercles in the animals treated with vitamin C. These findings could not be confirmed by Heise and Steenken [151], who state that vitamin C has no effect on the tuberculin reaction in tuberculous guinea-pigs or on the course of the disease in these animals.

Steinbach [818, 819] and his co-workers consider that the administration of vitamin C to tuberculous guinea-pigs increases the tolerance to repeated large doses of tuberculin, and that the simultaneous administration of the two substances gives better results than either substance alone. The results in chronic tuberculosis were better than in the acute form; fibrosis occurred more readily and caseation was less marked in the guinea-pigs receiving vitamin C. On the other hand, the course of experimental tuberculosis in animals such as rabbits, which synthesize their own vitamin C, was unaffected by the administration of the vitamin, with or without tuberculin. It is suggested that vitamin C might be used clinically to minimize the reactions to tuberculin.

The effect of vitamin C on diseases due to other organisms has also been investigated in the experimental animal. Thus Büsing [154] observed that the administration of vitamin C to rats or rabbits infected with pneumococci or staphylococci increased the survival time of the animals, compared with controls not receiving vitamin C.

Controlled clinical studies on vitamin C and resistance to infection have been made by Glazebrook and Thomson [816] on adolescents, who

normally received only 10 to 15 mg. of vitamin C a day in their food. Using one group of 1,100 as controls, another group of 385 was given large supplements of vitamin C daily. The incidence of the common cold and tonsillitis was the same in both groups, but whereas the duration of illness due to the common cold was the same in both groups, the duration of illness due to tonsillitis was longer in the control group than in the one receiving vitamin C. Cases of rheumatic fever and pneumonia occurred in the control group but not in the test group.

Kaiser and Slavin [189] have shown that there is a higher incidence of streptococcal infection of tonsils in children with low levels of vitamin C in their blood and tonsils than in controls with higher vitamin C levels. They also state that streptococci isolated from such children are seldom virulent. It has been reported that German children receiving a supplement of 50 mg. of vitamin C daily were less susceptible to infection than controls [876].

Recent well-controlled Scandinavian work, however, negatives any connection between vitamin C nutrition and the incidence or severity of respiratory infections (p. 568).

Bactericidal and Antitoxic Action. Vitamin C also possesses bactericidal and bacteriostatic powers. In suitable concentrations it prevents the development of pneumococci, streptococci, staphylococci [154], *Hæmophilus pertussis* [155], and *Clostridium tetani* [156] *in vitro*; at higher concentrations the organisms are destroyed. Thus in a concentration of 80 mg. per litre vitamin C inhibits the development of the *Hæmophilus pertussis*, while at a concentration of 1,000 mg. per litre it is definitely bactericidal. The vitamin C in blood cannot exert any direct bactericidal action, because of the relatively low concentration (optimum 12 mg. per litre). Any beneficial effect must be by stimulating other factors such as phagocytic activity. Pfannenstiel and Dötzer [815] conclude that the bactericidal titre of blood is independent of its vitamin C content. They also point out that the lowered vitamin C intake in Central Europe resulting from the war has had no deleterious effects on immunity.

Vitamin C also inactivates certain toxins, e.g., those of *Clostridium ædematens*, *Cl. histolyticus*, *B. dysenteriae* (Shiga) and *Clostridium tetani* [156-160]. Not only does the vitamin neutralize these toxins *in vitro*, but when given *in vivo* it is able to raise the resistance of animals against the toxin and acts curatively against doses up to 1 M.L.D. Certain strains of poliomyelitis virus are inactivated by vitamin C *in vitro*, and according to Jungeblut [161] it also has some curative effect *in vivo* with monkeys as subjects, although this is disputed by Sabin [162].

In vivo evidence in man conflicts with the animal observations. Spink and co-workers [169] gave vitamin C intravenously to human subjects with low vitamin C levels, but it did not result in an increase in the bactericidal action of the blood against coagulase-negative staphylococci, *E. coli* and *E. typhosa*. After oxidizing the vitamin C of human blood with copper no decrease in the bactericidal action against *E. coli*, *E. typhosa*, *Shigella paradysenteriae* and *B. flexneri* was observed.

A considerable amount of literature has been published on the effect of vitamin C on the toxin of diphtheria. Many workers have stated that

the degree of virulence of a standardized diphtheria toxin, as measured by the usual guinea-pig assay method, is dependent on the vitamin C intake of the animal, and that an increased intake of the vitamin gives increased protection to the animal [163-169]. Animals on a diet deficient in vitamin C are stated to survive half as long as animals with normal reserves receiving the same dose of toxin. It is also reported that vitamin C can inactivate the toxin of diphtheria *in vitro*. Willison [936] has recently investigated the question and she finds that there is undisputed evidence that oxidation of diphtheria toxin by vitamin C occurs under certain conditions. She emphasizes the importance of the pH and considers that the negative results obtained by some investigators are due to failure to observe this, to too short a period of incubation of vitamin C and toxin, and to an unfavourable ratio of vitamin to toxin. Willison states that 2 M.L.D. of diphtheria toxin is detoxicated by 1 mg. of vitamin C at 37° C.

According to Pakter and Schick [598] vitamin C has no effect on persons with a positive Schick reaction, although it neutralizes the toxin *in vitro*.

Jungeblut [817] has shown that the *in vitro* inactivation of diphtheria toxin by vitamin C occurs as a result of direct interaction between the two substances. Detoxication is enhanced by the presence of cupric ions, suggesting that the toxin is inactivated during the autoxidation of vitamin C. The substance responsible for the inactivation seems to be a peroxide according to Jungeblut. He observed that although vitamin C may protect guinea-pigs against a lethal dose of toxin, it fails to make subtherapeutic doses of antitoxin effective. Neither vitamins B₁ nor C had any demonstrable effect on the course of post-diphtheritic paralysis in guinea-pigs.

Zilva [170] and Torrance [171] and others [207, 208], have disputed these observations. Zilva claims that animals injected with vitamin C did not show any greater resistance to the diphtheria toxin than did the guinea-pigs which were depleted of vitamin C. Torrance has been unable to demonstrate any direct reaction between vitamin C and diphtheria toxin *in vitro*, nor has he been able to detect any significant differences in the vitamin C content of the suprarenals of test animals and controls injected with a sublethal dose of diphtheria toxin.

Vitamin C and Complement. There have been many attempts of recent years to correlate the complement activity of the serum and the vitamin C level in the blood. The most recent evidence negatives any direct relationship between the two. Zilva [176] and Chakraborty [469] found no significant alteration in the complement titre of scorbutic guinea-pigs, although Simola and Brunius [516] and Maish [178] claimed to have observed a lowering of complement titre in animals with vitamin C deficiency. Between 1938 and 1940 several reports were published establishing a direct correlation between the two. Thus Ecker and his colleagues [172, 175] state that *in vivo* there is a correlation at concentrations of vitamin C below 1 mg. per 100 c.c. of serum. They claim that in scurvy the administration of graded doses of vitamin C is paralleled by a corresponding increase in the complement titre. Chu and Chow [174] recorded similar

observations on thirty-eight patients on basal diets receiving increasing quantities of vitamin C.

More recent work has failed to confirm these observations, which were based on average results without statistical evaluation. Spink, Agnew and Michelson [855] in 1941 and 1942 showed that a fall in plasma vitamin C in both guinea-pigs and adult humans is not accompanied by a reduction in the complement titre, and that neither the *in vitro* nor *in vivo* addition of vitamin C results in change of complement titre. They further state that they have been able to remove vitamin C chemically from blood without changing the titre of complement. Kodicek and Traub [987] in 1943 were unable to find any significant change in complement in guinea-pigs partially or completely deficient in vitamin C. The method of titrating the complement was based on the determination of fifty per cent. hæmolysis and the results statistically evaluated. Kodicek and Traub also took experimental precautions to eliminate variables other than vitamin C intake that might influence the result. These observations are in agreement with those of Crandon and his colleagues [68] in human scurvy (p. 528), Feller and co-workers [856], and Natvig [214]. Deeny and his collaborators [988] carried out investigations on eighty patients suffering from acute infections in private practice in Ireland. The vitamin C and complement values of the blood were estimated, but they were unable to establish any linear relationship between the two in health or disease; when the two factors were plotted there was a random distribution of the points. There is apparently some relationship between vitamin C and acute infective conditions, but resistance to infection is not related to the level of vitamin C in the blood, provided there is enough to satisfy normal physiological needs. Further indirect evidence of lack of any correlation between vitamin C and complement titre is the observation that although newborn infants have a higher plasma vitamin C concentration than their mothers (p. 511), they have a significantly lower complement activity [987].

Feller and his associates [856] have also studied the following immunological phenomena in relation to vitamin C and also vitamin A nutrition :—

- (a) Capacity of nasal secretion to inactivate influenza virus.
- (b) Titre in blood serum of neutralizing antibodies for influenza virus.
- (c) Activity of lysozyme in the nasal secretion.
- (d) Phagocytic activity.
- (e) Complement titre of blood serum of polymorphonuclear neutrophile leucocytes in whole blood for pneumococci.

The results of the various immunological tests were not significantly influenced by marked changes in the plasma levels of vitamins A or C, or by a period of severe vitamin C deficiency followed by a large excess of the vitamin. The authors of the work point out, however, that because of the multiplicity of factors involved in the mechanisms of virulence, susceptibility and resistance broad conclusions cannot be drawn from the study.

Vitamin C and Leucocytosis. There is some evidence that vitamin C is taken up in large quantities by the leucocytes of the blood [182]. It has been demonstrated by special cytological methods by Tonutti and

Matzner [102, 103] in the leucocytes of the lungs in pneumonia. The latter found that in guinea-pigs with pneumonia the alveolar epithelium showed a mobilization of its cells to give a multiplication of the normal alveolar phagocytes laden with granules containing vitamin C. The alveolar exudate was full of cells also laden with the vitamin. According to Tonutti active phagocytes may contain 0.3 per cent. vitamin C. The presence of vitamin C in leucocytes may be associated with the increased needs of vitamin C in febrile states (p. 476). It would appear from the work of Cuttle [104] that considerable quantities of vitamin C are stored in the white blood cells, since in leukosis an excessive amount of the vitamin can be absorbed and retained and the amount in the blood cells is increased. These abnormalities bear a direct relationship to the number of circulating leucocytes. In leukosis whole blood contains more vitamin C than the plasma and the whole blood vitamin C falls below the plasma level coincidentally with the fall in the leucocyte count. The increased requirements for vitamin C in infections may be due to the accompanying leucocytosis. When erythrocytes and leucocytes compete for vitamin C *in vitro* it is taken up preferentially by the leucocytes [848].

Careful quantitative studies of variations in phagocytic power under different nutritional conditions have been made by Cottingham and Mills [989]. They have observed that vitamin C deficiency severe enough to retard growth produces a corresponding reduction in phagocytic activity. Thus the leucocytes of adequately fed guinea-pigs took up an average of 18.3 micro-organisms per cell *in vitro*, with ninety-nine per cent. of the cells showing evidence of bacterial destruction by the end of one hour. On a vitamin C deficient diet, phagocytosis was reduced to 7.3 bacteria per cell, with intracellular digestion reduced to seventy-four per cent.

It has been stated that the injection of vitamin C provokes a leucocytosis in infective states or in leucopenia [105, 106]. From histological studies on experimental fractures in rabbits, it would appear that the injection of vitamin C causes an increased proliferation of the reticulo-endothelial elements. This occurs even in animals on a diet containing an adequate supply of the vitamin [109]. Meyer [181] determined the opsonic index of three subjects and found that the injection of vitamin C caused a marked rise in the phagocyte count, in one case as much as six times the normal. The injection of doses of from 50 to 750 mg. of vitamin C into normal individuals causes considerable leucocytosis, *e.g.*, up to an increase of sixty-eight per cent. [854].

A reduction in the number of white blood cells occurs in scorbutic animals; the administration of vitamin C to these animals causes a temporary leucocytosis [178-180]. Crandon [68] also found that whilst on a scorbutic diet his white cell count fell from 5,000 to 3,500. After an injection of 1,000 mg. of vitamin C it rose to 5,000 and later to 9,000. Russian workers have found that a deficiency of vitamin C inhibits the function of the reticulo-endothelial system in guinea-pigs, and they suggest that this may be the reason for the lowered resistance to infection in scurvy [188]. Faulkner [184] observed that the administration of large doses of vitamin C in various infective conditions was accompanied

by appreciable reticulocyte responses analogous to those following the administration of vitamin C to patients with the anæmia of scurvy (p. 526).

In conclusion, whilst it is admitted that there is a case for believing that there is some connection between vitamin C and resistance to infection, much of the literature on the subject is inconclusive and not readily interpretable as it has been done under artificial conditions and on various species.

Vitamin C as a Detoxicating Agent. Arsphenamines. As pointed out by Ehrlich the most important means of detoxifying the arsphenamines is by chemical reduction. Vitamin C might be expected to occupy a unique position in this connection because it is a powerful reducing agent and is a normal constituent of living tissues. It was originally observed by Sulzberger and Oser [185] that large doses of vitamin C reduced and inhibited the susceptibility of the skin of the guinea-pig to experimental sensitization with neoarsphenamine. This was confirmed by Bertelotti [191] and by Cormia [186], who states that possibly sensitization does occur, but that vitamin C inhibits the cutaneous reactions. McDonald and Johnson [270] state that vitamin C has some protective action against arsphenamine reactions in guinea-pigs, but they could find no relation between the amount administered and the degree of protection. These results were questioned by Cohen [187], who in carefully controlled studies showed that there was no difference in sensitivity to neoarsphenamine between guinea-pigs on a diet deficient in vitamin C and those receiving an adequate supply. Chapman and Morrell [192] obtained results exactly opposed to those previously described; their guinea-pigs on a diet low in vitamin C were actually less sensitive to arsphenamine than those on a normal diet.

These discordant results are undoubtedly due to differences of technique. Thus Martin and Thompson [940] state that vitamin C is most effective in protecting mice against the toxic effects of neoarsphenamine if it is injected two hours before the latter, whilst McChesney, Barlow and Klinck [941] claim that the maximum protective effect is obtained if neoarsphenamine and vitamin C are injected in the same solution intravenously, and that if the vitamin C is given two hours before the neoarsphenamine its protective action is lost. They state that a dose of neoarsphenamine which represents LD_{50} kills only ten per cent. of the animals if this is injected with an equal weight of sodium ascorbate. The amount of vitamin C needed to exert a protective effect is between a quarter and an eighth of the weight of the arsphenamine or half a mole of vitamin C for a mole of neoarsphenamine. These investigators state that at a level of three moles of vitamin C per one mole of neoarsphenamine, the dose of the latter may be increased to 700 mg./kg. with no greater toxicity than that produced in controls receiving 400 mg./kg. If the vitamin C is injected simultaneously at another site the detoxifying action is somewhat decreased but not entirely eliminated.

Evidence for the increased tolerance of the human organism to the arsphenamines after vitamin C therapy is very conflicting. Dainow [188] claims that the tolerance for toxic drugs, including the arsphenamines,

depends upon the body reserves of vitamin C. He treats all cases of erythrodermia and toxic reactions due to parenteral arsenic therapy with oral or intravenous doses of 100 to 300 mg. of vitamin C daily, and he states that the period of treatment is considerably reduced. Lees [189] of Edinburgh gives vitamin C in oral doses of 300 mg. daily to avert the toxic effects of arsphenamine drugs given for the treatment of syphilis. Landfisch [190] similarly reports that he was able to treat twenty-five patients, who had previously shown definite sensitization to neoarsphenamine and marked symptoms of intolerance, by administering vitamin C with the neoarsphenamine or half an hour previously. Intravenous or intramuscular doses of 300 mg. a day controlled many of the symptoms of neoarsphenamine intolerance in twenty-two cases quoted by Welker [250]. Vail [822] describes spectacular relief from the symptoms of arsenic sensitivity by the use of relatively small doses of vitamin C (50 to 100 mg. daily) given in conjunction with arsenic medication. He states that the use of vitamin C does not lessen the therapeutic response to neoarsphenamine.

Large intravenous doses of vitamin C (500 mg. a day) followed by high maintenance doses of 100 to 200 mg. by mouth were given by Cormia [198] to patients who had previously suffered from neoarsphenamine dermatitis. He found that they were able to tolerate more of the same arsenical without further reactions, although he admits that his results were not so spectacular as those of Dainow. Actual cases of neoarsphenamine dermatitis cleared up within fourteen to eighteen days after giving 100–200 mg. of vitamin C by mouth. Cormia believes that vitamin C may possibly stimulate a local cellular immunity and overcome hypersensitiveness, or that it may act as a reducing agent, preventing the oxidation of arsphenamine into skin sensitizing antigens.

Falconer, Epstein, and Mills [194] studied a group of seven patients in whom attacks of thrombopenic purpura repeatedly occurred after the administration of neoarsphenamine and bismarsen. At no time and in none of the patients was any appreciable modification of sensitivity to the drugs observed during or after the administration of 100 mg. doses of vitamin C.

The blood vitamin C of a number of patients showing signs of sensitivity to neoarsphenamine was examined by Friend and Marquis [195]. The values were from 0.13 to 0.85 mg.—well below normal levels. It was concluded that a low blood vitamin C was the result of a toxic reaction to the neoarsphenamine rather than a predisposing factor to such reactions. This view is open to criticism because pre-reaction blood values were not determined, and four out of twelve patients receiving arsphenamine without reactions had low vitamin C blood levels. Farmer and his colleagues [196] have also demonstrated that patients hypersensitive to neoarsphenamine show very low vitamin C blood levels. In patients showing severe symptoms of intolerance a fall in the blood vitamin C occurred in spite of the oral administration of the vitamin during treatment. It was frequently observed that a marked lowering of the blood level followed the administration of neoarsphenamine in patients showing no intolerance to the drug. Delp and Weber [820, 821] also state that

patients showing sensitivity to arsphenamines have low vitamin C levels. By giving 800 mg. of vitamin C a day orally and 800 to 500 mg. intravenously every other day they claim that the sensitivity of such patients is diminished.

Lahiri [942] noted that the blood vitamin C is reduced in syphilis. He states that the administration of 800 mg. of vitamin C intravenously is helpful as a safeguard against the development of intolerance to arsenicals and improves liver function, as shown by liver function tests. If vitamin C has an action in reducing the toxicity of drugs such as the organic arsenicals it may be through the liver.

Bundesen [822] and his colleagues have carried out extensive observations on the detoxifying action of vitamin C in arsenic therapy. They have found that vitamin C prevents the *in vitro* oxidation of neoarsphenamine and mapharsen. A number of patients were patch tested with neoarsphenamine, and positive reactors retested with the drug to which vitamin C had been added. Not a trace of reaction was found in thirty-two out of thirty-eight who formerly reacted to neoarsphenamine alone. Further studies by Bundesen and his co-workers suggest that the majority of hypersensitive patients whose local cutaneous reaction to neoarsphenamine is fully prevented by vitamin C should be able to tolerate intravenous neoarsphenamine if the blood vitamin C is maintained at a sufficiently high level to inhibit the formation of toxic products of oxidation of the neoarsphenamine. This work has been confirmed and extended by Abt [215].

Beerman and his co-workers [942] claim that clinically the incidence of reactions of antisyphilitic arsenicals is reduced fifty-eight per cent. if the substances are dissolved in a one per cent. solution of methyl glucamine ascorbate before administration. White [216] has been unable to confirm this in animal tests.

Lead and Gold. It has been reported that vitamin C has a detoxicating action in cases of poisoning by other metals, such as gold and lead. Secher [197] states that thrombopenia and dermatitis, which sometimes develop in patients receiving gold therapy, can often be prevented by the administration of vitamin C. Sande [198] observed that the vitamin C content of the organs of guinea-pigs who had received injections of gold salts was much lower than that of control animals. Three patients after treatment with gold developed symptoms of intolerance and a lowered capillary resistance; one patient also had signs of liver injury. After treatment with 100 to 200 mg. of vitamin C a day given intravenously the symptoms of intolerance rapidly disappeared. Dainow [188] describes the treatment of erythrodermia due to gold therapy with intravenous injections of 0.2 gram of vitamin C.

Studies on lead poisoning were made by Holmes and his co-workers [199], who observed four hundred men exposed to lead over a period of a year. Thirty-four had symptoms of lead poisoning. Half were treated with additional doses of 100 to 200 mg. of vitamin C a day for several weeks, and in most the vitamin was said to be more effective in removing the symptoms of lead poisoning (irritability, insomnia, skin pigmentation, nervousness) and restoring the blood picture than in the

controls who received the usual therapy with calcium. Marchmont-Robinson [245], from a study of over three hundred lead workers concludes that 50 mg. of vitamin C a day protects them against the effects of chronic lead absorption.

Dannenberg [200] and Evans [948] with their co-workers are unable to confirm these observations. Evans and his colleagues studied a group of four hundred workers in a tetraethyl lead factory. The level of vitamin C nutrition was low. The administration of 100 mg. of vitamin C daily failed to have any effect on the lead concentration in the blood or on its elimination in the faeces and urine. No difference was noted in the physical condition, number and severity of complaints, erythrocyte count, number of stippled erythrocytes or haemoglobin percentage. Pillemer [201] in a well-controlled experiment with guinea-pigs found that in two series of forty-four guinea-pigs on a subclinical scurvy intake of vitamin C, the degree of lead poisoning that developed after a month's ingestion of lead carbonate was more severe in comparison with that in two groups of twenty-four animals saturated with the vitamin.

Frommel and Loutfi [1061] state that the electrocardiographic disturbances produced in guinea-pigs by the injection of bismuth sodium thiodiglycollate are diminished by the simultaneous injection of 0.1 gram vitamin C per kilogram.

Sulphonamides and Other Drugs. Claims have been made that the toxic effects of certain drugs can be prevented or minimized by the administration of vitamin C. Thus Dainow [202] and Bickel [208] state that the toxic effects of the sulphonamides (particularly sulphapyridine) can be prevented, or at any rate relieved, by the simultaneous administration of vitamin C in daily doses of 0.5 gm. given intravenously. Dainow has demonstrated that the urinary excretion of vitamin C is considerably decreased in patients receiving sulphanilamide. He also describes animal experiments in which large doses of sulphanilamide caused mental symptoms, jaundice and azoospermia and a fall in the vitamin C content of the brain, liver and testicles. It is argued that the normal vitamin C reserves are mobilized to detoxicate the sulphanilamide. Dunlop [204] has been unable to confirm Dainow's statement that vitamin C increases the tolerance of patients to sulphapyridine, and others have found no difference in the survival rate of rats and guinea-pigs treated with toxic doses of sulphanilamide alone and with sulphanilamide and vitamin C [825, 1058]. Perner [1014] gave full doses of sulphadiazine to fifty patients together with 100 mg. of vitamin C daily, and states that none of them suffered from any reactions. As no controls were observed it cannot be said that the vitamin C definitely exerted any protective action. Holmes [1088] observed an increased urinary excretion of vitamin C in patients given sulphathiazole, and recommended supplements of 100 mg. daily to allow for this. Shropp [1070] reports a case of sensitivity to sulphapyridine (red and swollen gums, soreness of mouth and eyes, pyrexia) that disappeared after giving vitamin C.

It has also been stated that vitamin C diminishes the toxicity of such substances as anaesthetics [218], benzene [205, 206], phosphorus [209], trichlorethylene, T.N.T. [828] and barbiturates [949].

There is some doubt about the detoxifying effect of vitamin C on T.N.T. Smith and his colleagues [950] were unable to observe any protective effect in cats, rats or guinea-pigs. In the United States a recommendation has been made that munition workers exposed to T.N.T. should receive at least 100 mg. of vitamin C daily [954]. Vitamin C appears to increase the rate of excretion of amphetamine (benzedrine) [252] and to increase the rate of metabolism.

Kimball [210] and Frankel [211] believe that the toxic effects of sodium diphenylhydantoin (soluble phenytoin), a drug recently used in the treatment of epilepsy, are more pronounced in patients deficient in

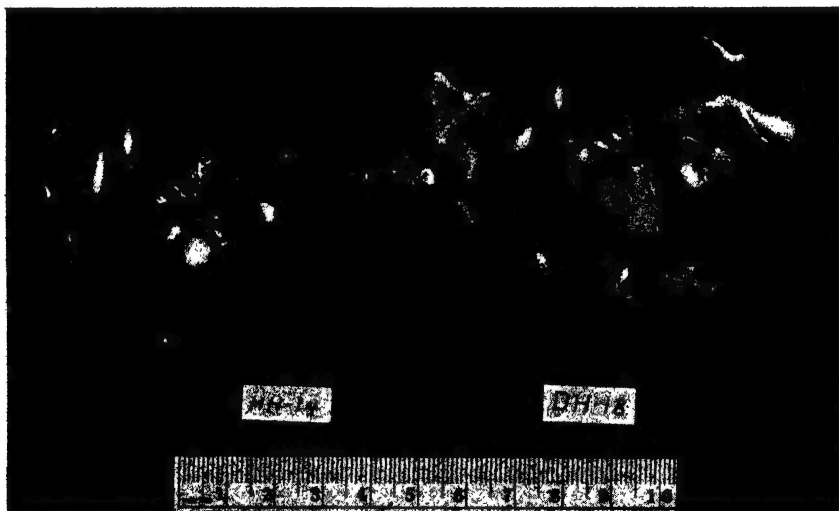


FIG. 138. The protective action of vitamin C against hepatotoxic agents. The illustration shows the livers of two guinea-pigs, both fed on a scorbutic diet for sixteen days before being given 25 mg. of hydrazine, a hepatic poison, for two days. Guinea-pig HH-14, whose liver is seen on the left, received daily injections of 80 mg. of vitamin C; guinea-pig DH-18, whose liver is shown on the right, did not. Macroscopically the liver of guinea-pig HH-14 was practically normal in appearance. The liver of guinea-pig DH-18, however, was pale, yellow, friable, and showed signs of fatty degeneration. It was sixty-eight per cent. heavier than the liver of guinea-pig HH-14.

vitamin C. According to Drake [599] in the experimental animal sodium diphenylhydantoin causes an increased excretion of vitamin C in the urine, and a lowering of the body reserves of the vitamin [599]. Gruhzit [212], however, found that the administration of this drug to animals had no effect whatsoever on the absorption and utilization of vitamin C, and others have reported that it has no effect on the vitamin C blood levels of patients [682]. An examination of epileptics receiving sodium diphenylhydantoin revealed that the plasma vitamin C was not influenced in any way by the type of therapy received, nor did the long continued administration of sodium diphenylhydantoin have any effect on the vitamin C level in the plasma. Gruhzit believes that the low levels of

PROTECTION OF VITAMIN C AGAINST LIVER TOXINS

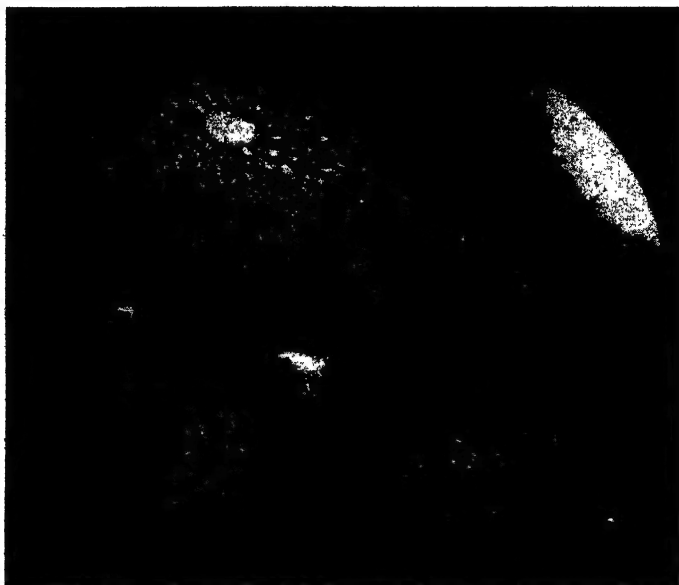


FIG. 134. The protective action of vitamin C against hepatotoxic agents. A section of the liver of guinea-pig HH-14 (see Fig. 133), given 30 mg. of vitamin C daily and then 25 mg. of hydrazine for two days. Hæmatoxylin and eosin stain ($\times 100$). The cellular appearance is almost normal. Under a magnification of $\times 400$ some hydropic changes in the cytoplasm are visible.

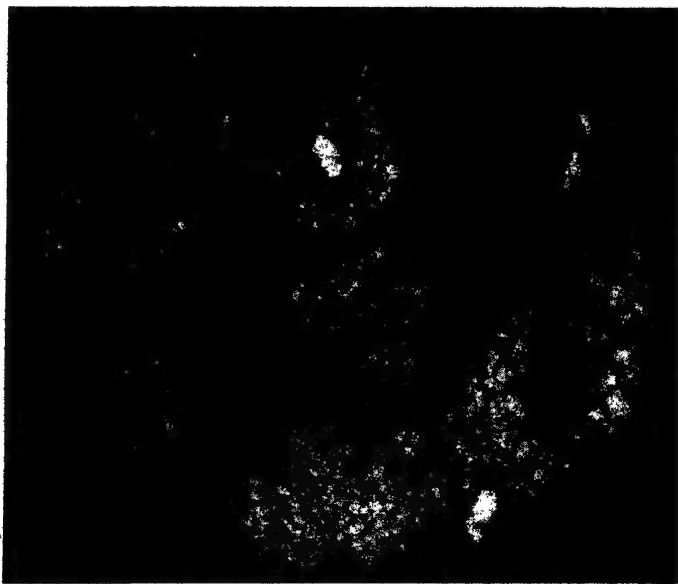


FIG. 135. The protective action of vitamin C against hepatotoxic agents. A section of the liver of scorbutic guinea-pig DH-19 (see Fig. 133) damaged by hydrazine. Hæmatoxylin and eosin stain ($\times 100$). Although the magnification is the same as in Fig. 134, the cells are increased in size and there is general loss of architecture and destruction of the cells and nuclei following fatty necrosis.

vitamin found in the plasma of epileptics receiving the drug is not due to the latter, but to an adequate intake of vitamin C. Ziskin [824] observed that gum hyperplasia resulting from the administration of diphenylhydantoin is not significantly altered by giving vitamin C.

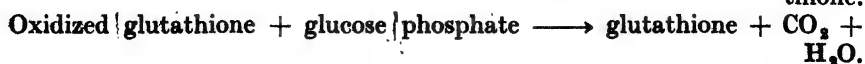
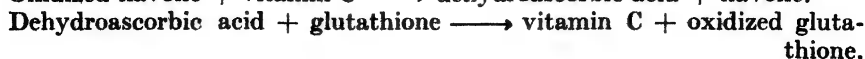
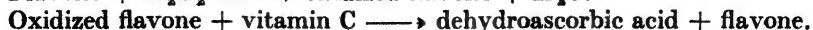
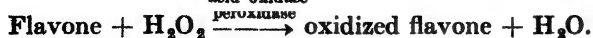
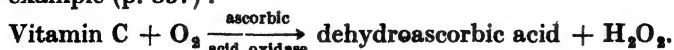
Other workers have more recently failed to confirm the earlier observations of Kimball and Frankel [945-948]. Emmett and co-workers [948] have conducted a careful investigation on the effect of phenytoin on the weight, gross pathological changes, plasma vitamin C levels and vitamin C content of the tissues of guinea-pigs and they concluded that the drug had no significant effect on the utilization of vitamin C in this animal.

Beyer [952] has shown that vitamin C protects against hepatotoxic agents such as hydrazine (Figs. 188 to 185). Guinea-pigs deficient in vitamin C showed an average of fifty per cent. more fat in their livers than guinea-pigs receiving adequate vitamin C when both groups were fed hydrazine. Gross and microscopic evidence confirmed the severity of fatty degeneration in the animals deficient in vitamin C.

Function of Vitamin C in Tissues. The rôle of vitamin C as an activator for the growth of tissues is well recognized. Thus Baker [464] found that it stimulates the growth of fowl monocytes, and others that it stimulates mouse sarcoma tissues [465]. It has a beneficial effect on the formation of fibres in fibroblasts in tissue culture [657]. Epithelial sheets of tissue cultures will not grow in media devoid of vitamin C [955].

It has been suggested that vitamin C is a component of a reversible oxidation-reduction system in the body, acting as a hydrogen transporter or respiratory catalyst. Attractive as this theory may be, there is no clear-cut evidence that vitamin C has this function in animal tissues, although experiments by Hopkins and Morgan [217] and by Borsook and his fellow-workers [218], strongly support the theory as far as plant tissues are concerned. The cytochrome-indophenol oxidase system has been shown to act as a catalyst in the aerobic oxidation of vitamin C, and such a system may be responsible for the slow oxidation of vitamin C in isolated animal tissues [219]. It has been argued that the oxidized form of the vitamin might be reduced by substances like glutathione [222]. Possible relationships between vitamin C and glutathione in oxidation-reduction systems have been demonstrated [217], and Prunty and Vass [958] have shown that the concentration of glutathione in human red blood cells varies inversely with that of the plasma vitamin C. The reduction of dehydroascorbic acid by animal tissues, such as liver, muscle and erythrocytes has been established [222].

Szent-Györgyi [660] has suggested that vitamin C takes part in a respiratory system involving the flavones, of which vitamin P is an example (p. 857) :—



**Demonstration of Vitamin C in Tissues by
Silver Staining Technique**

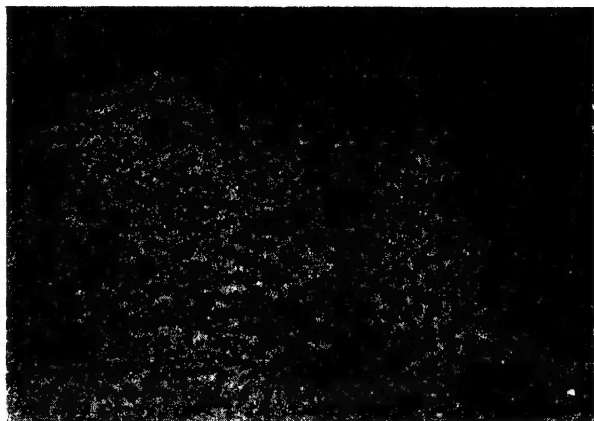


FIG. 136. Vitamin C in Embryonic Nerve Tissue. Vitamin C granules are seen scattered along a developing axone in a chicken embryo. Silver stain ($\times 800$).

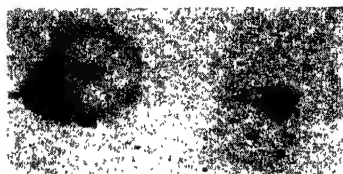


FIG. 137. Vitamin C in the Adrenal Cortex. Cells from the secretory zone of the adrenal cortex of the rat, showing vitamin C aggregated in the Golgi region of the cells. Silver stain ($\times 2,000$).

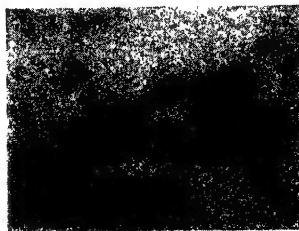


FIG. 138. Vitamin C in the Adrenal Medulla. Cell in the adrenal medulla of a cat, showing vitamin C aggregated in the nucleus near the Golgi region. Silver stain ($\times 1,200$).

Demonstration of Vitamin C in Tissues by Silver Staining Technique

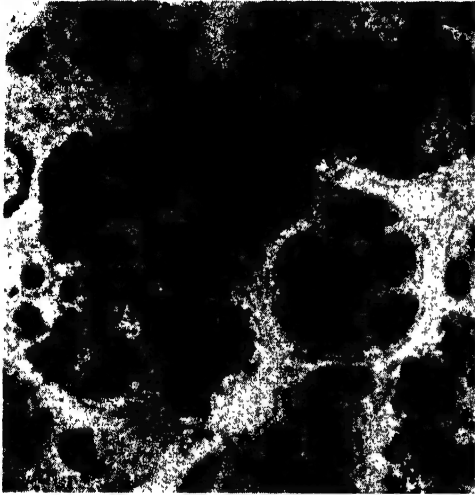


FIG. 139. Vitamin C in the Pituitary Gland.
Deposits of silver representing granules of
vitamin C in chromophil cells.



FIG. 140. Vitamin C in
Corpus Luteum of Dog.
After staining for fifteen
minutes with 0.4 per cent.
silver nitrate solution.



FIG. 141. Vitamin C in the Pituitary of a Dog.
After staining for fifteen minutes with 0.4 per
cent. silver nitrate solution.



FIG. 142. Vitamin C in Interstitial
Cells of Testis. After staining for
fifteen minutes with 0.4 per cent.
silver nitrate solution.

On the other hand, a strong argument against the theory that vitamin C functions as a major respiratory catalyst is the observation of Stotz and co-workers [220] that tissues such as liver and kidney obtained from scorbutic animals do not show a decreased respiration capacity despite their low levels of vitamin C, and that when vitamin C is added to the depleted tissues there is no appreciable rise in the oxygen consumption. This is at variance with the observation of Harrison [254], Euler and Klussman [255], that slices of fresh scorbutic tissue have a low oxygen uptake that is restored by adding vitamin C *in vitro*.

King [221] and his co-workers were unable to show that vitamin C acted as a hydrogen transporter in an *in vitro* system containing nicotine, hæmochromogen, vitamin C, coenzyme, glucose dehydrogenase and glucose. This would suggest at any rate that vitamin C does not function as a respiratory catalyst in those dehydrogenase systems which depend on cozymase as a coenzyme. Several investigators have suggested that the dehydrogenation of glucose might be accomplished in a system in which vitamin C functions as a carrier [228]. Others state that the oxidation of certain fatty acids proceeds at a higher rate in the presence of vitamin C [224].

Independently of, or parallel with, the possible function of vitamin C as a carrier-catalyst in tissue respiration, the vitamin may be of importance in cellular physiology as a regulating and protecting agent. Thus Harrer and King [554] have shown that in scorbutic guinea-pigs the activity of liver esterase and phosphatase is reduced, and that there is a marked drop in the activity of respiratory enzymes.

Vitamin C and Hormones. The Adrenals. The work of Bourne [227], Gough [887] and Giroud [228] on the cytological demonstration of vitamin C in tissues by staining with silver nitrate has shown that the vitamin is particularly abundant in the corpus luteum, the pituitary, the adrenals and glandular tissue (Figs. 137 to 142). Other workers have commented on the singularly high concentration of vitamin C in certain physiologically related endocrine organs—the adrenal cortex, corpus luteum, and the anterior lobe and pars intermedia of the pituitary [229].

The finding of large quantities of vitamin C in the adrenal medulla and cortex (see Figs. 137 and 138), and its supposed association with oxidation-reduction processes in tissues, suggested that it played an important rôle in the metabolism of the adrenals. It is located in the region of the Golgi apparatus of those cells of the adrenal cortex (Fig. 137) in which the biologically active 17-ketosteroids are present [288]. The view has been advanced that vitamin C serves to stabilize both the medullary hormone (adrenaline) and the cortical hormone [230]. It is further suggested that it is concerned in the regulation of skin pigmentation. Thus Szent-Györgyi [281] wrote as far back as 1930: "Hexuronic acid (*i.e.* vitamin C) completely inhibits the formation of pigment in all systems in which a melanoid pigment is formed through the oxidation of a phenol. The absence of hexuronic acid in Addison's disease could thus give a clear explanation of the mechanism of pigment formation." Abderhalden [282] has shown *in vitro* an inhibition of pigment formation from adrenaline and from *l*-3:4-dihydroxyphenylalanine ("dopa") by vitamin C both with

and without the addition of the enzyme tyrosinase. Schröder [233] and others [234], using slices of guinea-pig ear, have shown inhibition of pigment formation from dopa in the presence of vitamin C.

It was suggested in 1940 by Giroud and others [1081] that the synthesis of the cortical hormone is dependent upon the presence of vitamin C in the adrenals. More recently Sayers and his co-workers [1082] have produced further evidence in favour of this. They observed a fall in the vitamin C and cholesterol content of the adrenals of rats after injecting the adrenotropic hormone. This fall reached its lowest level in one hour, remained there for two to three hours, returned to its original level after nine hours, and finally after twenty-four hours reached a greater level than in control animals. The authors suggest that the concentration of both the cholesterol and the vitamin C in the adrenals falls after the injection of the adrenotropic hormone because they are essential for the synthesis of the cortical hormone.

Some workers suggest a clinical relationship between vitamin C and the adrenal gland. Morawitz [235], Hoff [236] and Rothman [872] claim that the pigmentation of Addison's disease may be strikingly diminished by the oral or parenteral administration of vitamin C. Wilkinson and Ashford [237] found a vitamin C deficiency, as judged by a low urinary excretion, in three patients with Addison's disease, and they observed an increase in the excretion of the vitamin after the administration of 800 to 500 mg. a day. When therapy was discontinued the increased excretion fell rapidly to values previously observed. A definite parallelism between the degree of vitamin C deficiency and the severity of the disease was recorded. They expressed the view that depletion of vitamin C might be a feature of Addison's disease or it might be an entirely non-specific index of the extremely low state of health of the patients. Similar findings were reported by von Drigalski [238] and Siwe [239]. The latter found a high tolerance for vitamin C in patients with Addison's disease; thus urinary excretion was not increased after the oral administration of 600 mg. a day. Although there was a decrease in pigmentation, the symptoms of the disease were not relieved.

Rothman [872] noted a bleaching of the pigmented areas in Addison's disease after giving 500 mg. of vitamin C daily by mouth for six months; the effect was easily recognized in coloured photographs. But the patient was not suffering from a deficiency of vitamin C before treatment, so that the disease could not be directly associated with this. Rothman has shown that melanin is reduced by vitamin C to water-soluble lighter pigments and he attributes the diminution of hyperpigmentation in Addison's disease by vitamin C to this effect. He has also shown that vitamin C furthers the actinic transformation of tyrosine into "dopa," but inhibits any further oxidation if excess is constantly present.

Sendroy and Miller [240] studied the combined urinary clearances of urea and vitamin C in eight patients with nephritis, and they concluded that a relationship existed between renal efficiency as indicated by the clearance of urea and the amount of vitamin C excreted. They suggested that in Addison's disease, in which a renal insufficiency exists, the deficiency in the vitamin C excretion observed by earlier workers is

caused by renal insufficiency rather than by any relationship between vitamin C and the adrenal hormones. Blood plasma values for vitamin C as well as urinary excretion in Addison's disease were determined by Jenovese [241], who found that although the urinary excretion of vitamin C was low the blood plasma values were normal, suggesting that urinary excretion alone cannot be used as an index of vitamin C deficiency in Addison's disease. Pojer [242] treated two cases of Addison's disease with vitamin C and noted an improvement in the general condition and a reduction in the pigmentation.

It is also stated that there is not only a relationship between the adrenal cortex and vitamin C, but also between the latter and the medulla. Thus the administration of vitamin C is stated to increase the adrenaline content of the adrenals of normal guinea-pigs [244].

Thyroid. Several observers have pointed out a possible relationship between vitamin C and the functions of the thyroid gland. Demole and Ippen [258] consider that vitamin C antagonizes thyroxine. They found that the loss in weight of a scorbutic guinea-pig could be checked by the administration of 0.5 mg. of vitamin C a day. The injection of 0.1 mg. of thyroxine, however, caused a loss in weight that was only checked by raising the dosage of vitamin C to 10 to 20 mg. Doses of thyroxine that were normally fatal were also tolerated by the animals if they were given large doses of vitamin C. Similar observations were made by Belasco and Murlin [243]. Marine [259] observed that vitamin C prevents thyroid hypertrophy in guinea-pigs injected with the thyrotropic factor of the anterior pituitary. Spence and Scowen [260], however, were unable to confirm this work. Sure and Theis [261] state that if toxic doses of thyroxine are fed to rats a reduction in the vitamin C content of the adrenal, thymus and kidney occurs. Paal and Brecht [262], however, observed a rise in the vitamin C content of the adrenals and liver of rats receiving thyroxine. Heyl [263] also states that the histological changes in the thyroid produced by large doses of vitamin C simulate those produced by thyroxine.

Clinical observations have been made on the excretion of vitamin C in hyperthyroidism by Lewis [264] and von Plehwe [265]. Lewis discovered that in thyrotoxic patients awaiting operation the excretion of vitamin C was far below normal, and only reached normal levels after thyroidectomy. Following a control period von Plehwe gave himself injections of 2 mg. to 4 mg. of thyroxine daily. The pulse rate and urinary creatine increased, and the basal metabolism increased by fifty per cent. At the same time there were definite subjective symptoms such as headache, nervousness, sweating and tremor. Daily injections of from 500 mg. to 1,500 mg. vitamin C were then given while continuing the thyroxine. There was a noticeable improvement in the subjective symptoms and a prompt drop in the creatinuria, although the basal metabolic rate was unaltered.

Corpus Luteum and Ovary. The corpus luteum is another endocrine body rich in vitamin C [266]. Its demonstration by the silver staining technique is shown in Fig. 140. Biskind and Glick [229] showed that the vitamin C content of the corpus luteum falls as the organ atrophies.

They suggest that there may be a connection between the presence of vitamin C and the corpus luteum hormone progesterone. Thus the vitamin C content of the corpus luteum appears to run parallel with the progesterone content [267]. It has also been stated that in the scorbutic animal degeneration of the corpora lutea and failure of normal gestation result [268], although Bourne [266] has been unable to confirm any interference with pregnancy in the scorbutic guinea-pig. Pillay [728] estimated the urinary excretion of vitamin C of eleven women through twenty-four menstrual cycles and obtained some evidence that there was a decreased excretion of vitamin C at the time of ovulation. Bourne [269] believes that vitamin C is associated non-specifically with the production of the corpus luteum.

Israel and Meranze [826] state that vitamin C has a progesterone-like effect on the rabbit endometrium. According to them the endometrium of rabbits treated with vitamin C approximated, particularly microscopically, to the secretory changes in the endometrium of control animals receiving progesterone. Pratt [958], however, was unable to confirm this.

The activity of the gonadotropic hormones is also said to be potentiated by vitamin C. The simultaneous injection of gonadotropic hormone and vitamin C for twenty to thirty days to immature rats produces a greater increase in the development of the male and female genitalia than does the hormone alone [857].

Vitamin C and Carbohydrate Metabolism. There appears to be some relation between vitamin C and carbohydrate metabolism, but from the mass of conflicting observations the exact relationship is not clear. In the vitamin C deficient animal glycosuria, a diabetic type of glucose tolerance curve, and depletion of the glycogen content of the liver have been reported by Banerjee [954], who also showed that the insulin content of the pancreas is markedly diminished in scorbutic animals. King [246, 247] has also shown that if guinea-pigs are depleted of vitamin C and given diphtheria toxin the islands of Langerhans degenerate, the blood sugar is elevated and the glucose tolerance is lowered. It is difficult to know whether this effect was due to deprivation of vitamin C or to the effect of the diphtheria toxin. Liver glycogen is also stated to fall in guinea-pigs on a diet deficient in vitamin C [664]. Hjörth [665] noted that the glucose tolerance curve was higher in individuals on a low intake of vitamin C than when they were given adequate vitamin C. This was confirmed by Secher [668] who obtained a diabetic type of glucose-tolerance curve in subjects with a low blood vitamin C; the curve was restored to normal type on giving vitamin C. Secher failed, however, to affect significantly the glucose-tolerance curve of diabetics by giving them vitamin C.

Observations have also been made on the effect of vitamin C on the blood sugar level of normal persons. Stepp, Schröder and Altenburger [248] gave 300 mg. of vitamin C intravenously to normal persons and recorded a considerable fall in blood sugar, *e.g.*, of twenty to fifty per cent. A similar observation was made by Sylvest [669], but the magnitude of the fall bore no relation to the vitamin C level of the blood. According to

Stepp vitamin C is ineffective given by mouth. Other workers have failed to reproduce these results [670]. Indeed, some have observed a rise in the blood sugar after administering vitamin C [459].

Tests have also been made on diabetics. Pfleger and Scholl [249] reported that the administration of vitamin C to diabetics who were not given insulin had no effect on the glucose level of the blood or urine, but it reduced the amount of acetone bodies in the latter. When, however, the tissues were saturated with vitamin C the action of insulin was perceptibly intensified, so that smaller amounts were needed to control the blood sugar. Similar observations were made by Hamne [827]. The patients were also stated to improve in their general condition. Stöger [251] studied the renal threshold for glucose in normal and diabetic subjects, and he observed that large doses of vitamin C altered the renal threshold for glucose in diabetes and rapidly reduced the glycosuria.

Bartelheimer [253] noted that the state of vitamin C nutrition of diabetics compared favourably with that of non-diabetic patients, a finding that has been confirmed by Sebesta [256] and his co-workers. Bartelheimer, however, observed a distinct lowering of the blood sugar when diabetics were fully saturated with vitamin C, whether insulin was given or not, but it had little effect on the blood sugar values after the ingestion of large doses of glucose.

Owens and his collaborators [257] have studied the vitamin C nutrition of a number of diabetics and observed that sixty-six out of a hundred had a low plasma vitamin C. This is not surprising, as all their patients came from a charity hospital. Although they agree that a good intake of vitamin C is advantageous, no concrete evidence was obtained that it had any effect on the state of severity of the diabetes when given in large doses of 300 mg. to 1,200 mg. daily. Nor was any constant objective improvement noted as a result of administering large doses of the vitamin. As many patients with a high level of vitamin C were taking insulin as those with a low level. Patients with adequate vitamin C nutrition had glycosuria and hyperglycæmia just as frequently as those inadequately nourished, and the low levels of vitamin C were not related to infection and cataract. A further finding was that the state of diabetic control bore no relationship to the blood vitamin C, nor did the administration of insulin in any form or dosage have any effect on the vitamin C status of the diabetic patient.

These observations on vitamin C and carbohydrate metabolism need careful interpretation. Many of them have been made on the immediate effects of the blood sugar level following an intravenous dose of vitamin C or on the effect of vitamin C on diabetics who have recently come under control. A change in blood sugar following the administration of vitamin C is no evidence of a direct relation between the latter and carbohydrate metabolism, because the change may be due to an alteration in the renal threshold for glucose or to some other temporary effect on storage mechanism. The fact is also disregarded that the tolerance of diabetics improves with hospitalization and the beneficial results obtained are often ascribed to any new form of therapy.

There are indications that vitamin C may be concerned with the rate

of disappearance of the blood lactate that accumulates during the performance of physical work. Crandon [274], who induced experimental scurvy in himself, noted impairment of his ability to perform aerobic work, *e.g.*, walking and running on the treadmill, and running to the point of exhaustion. Fatigue and weakness, as judged by performance tests, became progressively worse after the third month on the scorbutic diet, and these findings were correlated with an abnormally low rate of disappearance of blood lactic acid produced by the work. Lactic acid is stated to accumulate in the muscles of animals deficient in vitamin C [254]. No great change, however, could be found by Crandon in the capacity for performing anaerobic work, *e.g.*, in the muscular activity of the forearm as measured by an ergograph. The observations of Crandon on anaerobic work do not entirely agree with those of Basu, Biswas and Ray [275, 276]. They report that vitamin C, in such concentrations as are known to occur in the body, augments the contractions of skeletal muscle induced by single and repeated stimuli, brings about quicker relaxation and delays the onset of fatigue. They also state that vitamin C augments the rhythmic contractions of the isolated heart. Studies were later made on the muscular performance of human beings. Using an ergometer fatigue curves of the finger muscles were studied before and after saturation with vitamin C, and it was concluded that the contractility and fatiguability of human muscle depend upon the degree of its saturation with vitamin C. The work of Basu and his fellow-workers on human muscle is open to criticism, however, since it is difficult to rule out the psychological factor in human fatigue tests, and no control experiments were done.

Other Functions ascribed to Vitamin C. From the high vitamin C content of active tissues it is considered that vitamin C is essential for synthetic processes in the cell. The histochemical work of Bourne [271] has demonstrated that vitamin C is contained in the Golgi apparatus of the cell and in the mitochondria. His results have emphasized the selective retention of the vitamin in the active parts of the cell and in vital tissues. It is claimed that whenever a cell is synthesizing quantities of material the Golgi apparatus absorbs vitamin C [889]. Thus Barnett and Bourne [840] have demonstrated that in the histogenesis of the chick embryo, differentiating cells show a high concentration of vitamin C in the Golgi apparatus. The latter is recognized as the synthesizing centre of the cell, and the existence of vitamin C in it during active synthesis suggests that it may play a part in the synthesis of a variety of biochemical compounds. It has also been shown that the Golgi apparatus suffers in experimental scurvy and returns to normal when vitamin C is given [956]. Tonutti [102] states that vitamin C may appear in large quantities in active cells, which contain very little of the vitamin in the resting state. Thus there is very little vitamin C in ordinary muscle fibres but the muscle forming cells in the embryo contain relatively large amounts. The cells of the membrana granulosa of the Graafian follicle are also practically free of vitamin C, yet the corpus luteum contains a considerable quantity. Normally vitamin C cannot be demonstrated in any concentration in the adrenal medulla. On exciting the splanchnic nerves, however, adrenaline is released, and at the same time the cells of the medulla become charged

with vitamin C granules, although there is no change in the cortical cells, which do not elaborate adrenaline.

Does vitamin C play a part in intermediate protein metabolism? Leucine, tyrosine, and glycine are eliminated in the urine in considerable quantities after poisoning with hepatotoxins such as phosphorus. Roman-yuk [958] has shown that the cathepsin activity of kidney, liver and muscle from guinea-pigs suffering from vitamin C deficiency is increased. Alkaptonuria, a condition in which homogentisic acid is excreted and makes the urine almost black, may be produced in animals and man by the administration of the amino-acid tyrosine on a diet low in vitamin C [225]. The homogentisic acid disappears from the urine, which becomes normal in colour, if vitamin C is given in moderate excess, even if the administration of tyrosine is continued. This is an *in vivo* example of vitamin C bringing about the oxidation of tyrosine, which is an intermediate product of protein metabolism. Further observations by Law and Sealock [1067] prove that vitamin C can oxidise tyrosine *in vitro* and *in vivo*.

Recent work suggests that vitamin C is essential for the metabolism of certain aromatic amino acids in the growing infant. Levine [272] and his co-workers gave premature infants diets of cows' milk containing 5 grams or more of protein per kilo but no vitamin C. They observed a spontaneous defect in the metabolism of aromatic amino-acids, manifested by the excretion in the infants' urine of *l-p*-hydroxyphenyl-lactic and *p*-hydroxyphenylpyruvic acids. This defect was observed as early as the sixth day of life and persisted as long as vitamin C was withheld from the diet. The administration of vitamin C caused the disappearance of these acids without necessarily raising the plasma vitamin C levels. Full term infants showed this error of metabolism when given phenylalanine or tyrosine, and as in the case of premature infants it was corrected by the administration of vitamin C.

Stewart [278] claims that vitamin C may affect the cycle of changes undergone by visual purple in the process of dark adaptation (p. 62). He states that daily doses of 150 mg. of vitamin C produce as great an improvement in dark adaptation as daily doses of 24,000 units of vitamin A in subjects with poor adaptation. He believes it is possible that vitamin C aids the utilization of vitamin A. Yudkin [880] has been unable to confirm these observations.

Absorption of Vitamin C. According to Zilva [277] vitamin C is selectively absorbed by the tissues of the intestinal tract, chiefly by those of the small intestine. Ludden [882] and his colleagues showed that even a daily oral dose of 700 mg. of vitamin C was insufficient to maintain the normal vitamin C level in a patient who had most of the small intestine resected. This proves that vitamin C is primarily absorbed from this organ. From intubation studies Nicholson and Chornock [957] found that the degree of absorption of vitamin C from the intestine is independent of the degree of saturation or even of the magnitude of the absorptive surface, but depends rather on the concentration of vitamin C in the intestinal medium. According to Chinn [281] the absorption of vitamin C from the intestine is governed by simple physico-chemical laws. He states that in

the case of the rat from fifty to sixty per cent. of the ingested vitamin C is absorbed from the gastro-intestinal tract, the absorption being the result of a simple diffusion mechanism, in which the rate of absorption varies directly with the concentration of the vitamin C administered.

Certain strains of bacterial flora appear to be able to decompose vitamin C *in vitro*, but whether they exert this effect in the intestine is open to criticism. Stepp [278] and Einhauser [279] showed that certain strains of *E. coli* and *B. paratyphosus* were capable of destroying vitamin C. Kendall and Chinn [280] showed that certain bacteria isolated from the stomach and intestinal contents were able to ferment the vitamin. They noticed that the presence of even small amounts of glucose definitely prevented the bacterial destruction of vitamin C, as it was utilized preferentially, and since glucose is present in many foodstuffs, it is possible that it prevents the bacterial decomposition of vitamin C in the intestine. Young and his co-workers [959] have shown that numerous strains of enteric organisms, including *E. coli*, *Salmonella*, *Eberthella*, intestinal streptococci, vibrios, and *Proteus morgagni* can decompose vitamin C. While normally this may be of no significance in some pathological conditions it might account for a clinical deficiency of vitamin C even if the intake is adequate. According to Wilder and Wilbur [282] variable amounts of vitamin C are lost in the stools or destroyed in the gastro-intestinal tract, even under normal conditions. Gould and Shwachman [960] conclude that there is a loss of about twenty-seven per cent., presumably somewhere in the intestinal tract, when vitamin C is taken by mouth.

Farmer, Abt and Chinn [284] have developed a special technique for the estimation of vitamin C in faeces [285], and have found that the normal faecal excretion is very low—not over 5 mg. a day—and even with massive oral doses of 1,000 mg. a day does not exceed 15 mg. Similar findings have been reported by others [291]. Farmer and his colleagues believe that in health the absorption of vitamin C from the human intestine is almost complete, since on normal intakes faecal excretion is negligible. They obtained no evidence of phosphorylation as a requirement for its absorption through the intestinal mucosa, as has been suggested for riboflavin (p. 821).

It has long been known that patients suffering from gastro-intestinal diseases excrete subnormal amounts of vitamin C in their urine. This has been attributed in the past to a low intake resulting from a restricted diet, but the work of a number of investigators suggests that defective absorption may be a more important factor in some cases. Under abnormal conditions of the gastro-intestinal tract associated with abnormal bowel motility (catharsis, diarrhoea, ulcerative colitis) marked losses of vitamin C taken by the mouth may occur. Thus these conditions are frequently severe enough to decrease the blood level of vitamin C as well as the excretion in the urine, even when large amounts of the vitamin are given by mouth [286]. On recovery the blood plasma levels and the urinary excretion of vitamin C return to normal if the intake is adequate. When vitamin C is given parenterally to children with severe diarrhoea, a rise in the blood plasma level and the urinary excretion results, in contra-

distinction to the lack of response obtained if the vitamin is given to such children orally [286].

Abt and his fellow-workers [287] have studied the effect of catharsis and diarrhoea on the gastro-intestinal absorption of vitamin C in infants. A normal ten-months-old infant was placed on a metabolism frame, and the blood plasma level, and the faecal and urinary excretion of vitamin C determined. Even with large oral supplements the faecal excretion remained below 4 mg. a day. After giving 4 grams of Epsom salts, which resulted in the passing of semiliquid stools, there was a tenfold increase in the faecal excretion of vitamin C. Observations on a number of infants suffering from acute non-specific diarrhoea showed that the ingestion of large amounts of vitamin C was followed by a greatly increased faecal excretion, a low urinary excretion and a low blood plasma value. Up to a quarter of the oral dose was found in the faeces. The faecal excretion of vitamin C returned to normal when the diarrhoea disappeared [881]. Wright and Ludden [288] also report that in the presence of achlorhydria or diarrhoea signs of vitamin C deficiency have been found to appear in spite of a liberal intake.

Abt [478] and his co-workers state that vitamin C is inadequately absorbed in patients with achlorhydria. Forty-four patients with this condition associated with pernicious or iron deficiency anaemia certainly showed low blood levels of the vitamin, and in the case of pernicious anaemia the level could not be raised to normal by an adequate intake. It is possible that hydrochloric acid is necessary for the absorption of vitamin C. The administration of alkalis to gastric ulcer patients does not appear to interfere with absorption [832].

Supplements of vitamin C are probably better given with or just after meals, as there is some evidence that absorption is more complete when the vitamin is taken with food [1048].

Storage and Distribution of Vitamin C in the Body. A certain amount of vitamin C must be stored in the body because human scurvy takes several months to develop on diets deficient in the vitamin. It is found in practically all the tissues and body fluids. Muscle contains about 2 mg. per 100 grams, and although the vitamin is not fat-soluble it is stated to be present in stored fat. Glandular tissue, particularly the adrenals, pituitary, corpus luteum and thymus contain appreciable quantities, the concentration decreasing successively in the liver, spleen, testis, ovary, brain, thyroid, kidney, lung and heart. The adrenals are the richest organs in vitamin C (100–200 mg. per 100 gm.). There is a high concentration in the intra-ocular fluid, ciliary body, iris and lens [961, 962]. It appears that vitamin C is stored in those organs and tissues with a high metabolic activity, and is present to a greater extent in the tissues of the young. It is interesting to note in this connection that tumour tissue has been found to have a high vitamin C content during periods of rapid growth [289], and that patients suffering from cancer excrete low levels of vitamin C [290].

Vitamin C is present in the blood, cerebrospinal fluid, saliva [292], aqueous humour [293], and gastric juice [294]. Plaut and Bülow [295], who have observed a direct relationship between the vitamin C content

of the cerebrospinal fluid and the dietary intake, claim that determinations on the cerebrospinal fluid may supply the best index of vitamin C nutrition. This has not been confirmed by other workers. Wortis [296] and his co-workers, in a study of a hundred and thirty-three normal persons, who had received a test dose of 1 gram of vitamin C, found values ranging from 1.82 to 4.18 mg. per 100 c.c., with an average value of 3.05 mg. Much lower values were obtained by Jetter and Bumbalo [297], who obtained figures varying from 0.7 to 2.1 mg. per 100 c.c., but the state of vitamin C nutrition was not so carefully controlled as in the group studied by Wortis. There appears to be a seasonal fluctuation, with maximal values in the autumn and minimal values in late spring and early summer [298]. An average of 2.7 mg. per 100 c.c. is given by Castex and Schteingart [299], with 2.1 mg. in pathological conditions of the central nervous system, a difference that is not statistically significant. The quantitative variations in the cerebrospinal fluid in diseases of the central nervous system were inconstant and not related to the disease or to any alterations in the fluid. Many observers have stated that the level of vitamin C in cerebrospinal fluid is low in old age, and since oral administration restores it to normal levels these low values in old people may be due to lowered requirements because metabolism is slowed in the aged [296, 300].

In normal subjects the vitamin C content of the blood plasma ranges from 0.6 to 2.5 mg. per 100 c.c. [302]. After the injection of large doses of vitamin C, *e.g.*, 1 gram, the plasma level may reach a figure of 250 mg. per 100 c.c.; fifteen hours later it falls to about 20 mg. and returns to normal in two days [968]. According to Heinemann [301] the concentration of vitamin C in the blood cells consistently exceeds that in the serum or plasma. Since the vitamin is taken up slowly from the plasma by the red blood cells, its concentration in the plasma may exceed that found in the cells shortly after administering vitamin C. van Eekelen [303] and Schneider [304] state that the distribution between cells and plasma is approximately equal. Several workers have noted that the plasma vitamin C falls during pregnancy and lactation, owing no doubt to the increased needs of the foetus [305].

Vitamin C is secreted in the milk. The amount in human milk, which varies from 1 to 10 mg. per 100 c.c., depends upon the dietary intake of the mother. The values taken as a whole show that breast milk from women on diets satisfactory with respect to vitamin C contains from 4 to 8 mg. per 100 c.c. during the first few months of lactation [405-409]. According to some authorities a concentration below 4 mg. per 100 c.c. indicates a vitamin C deficiency in the tissues of the nursing mother [305]. Neuweiler [309] states that the vitamin C content of breast milk cannot be made to increase beyond a certain maximal value, however great the intake of the mother.

Excretion of Vitamin C. Vitamin C is excreted in the faeces, urine and sweat. Normally the faecal excretion is very low, usually of the order of 5 mg. a day [284, 285], although as pointed out by Chinn [285] considerable quantities of vitamin C may be passed with the faeces in diarrhoea and other gastro-intestinal disorders, *e.g.*, mucous colitis. Large variations

in the dietary intake have scarcely any effect on the faecal excretion of the vitamin.

Bernstein [811] originally stated that up to 2 mg. of vitamin C an hour could be lost in sweat under conditions of strenuous exercise. Others have been unable to verify this and consider that the loss is negligible or even nil [812, 968-966].

The urinary excretion of vitamin C depends on the intake [818]. When it is withdrawn from the diet its excretion falls progressively. If large quantities are administered to a person whose supplies are depleted, the urinary excretion does not rise to normal levels at once. Only small amounts continue to be excreted in the urine, while a steady rise in the plasma level occurs. If the administration of vitamin C is continued long enough, the tissues ultimately become "saturated" with respect to vitamin C and a rapid rise in the urinary excretion occurs. The urinary excretion is dependent on the concentration in the blood plasma. Like glucose and some other compounds present in the blood it is a "threshold" substance. The renal threshold, or level at which vitamin C leaves the blood and passes through the kidney into the urine, has been calculated by Lewis [967], who gave supplements of vitamin C in graded amounts until the urinary vitamin C excretion began to rise sharply; the vitamin C level of the blood when this occurred was taken to be the renal threshold. It varied from 1.1 to 1.8 mg. per 100 c.c. of plasma, with most of the values falling between 1.1 and 1.3 mg. This agrees with the figure of about 1.4 mg. given by other workers [814]. According to Crandon [68] the urinary threshold may be much lower, *e.g.*, 0.85 mg. per 100 c.c. in a state of severe vitamin C deficiency.

Retention of vitamin C does not occur once the body is saturated with the substance. The urinary excretion of the vitamin does not follow its rate of absorption from the alimentary canal, but depends on its rate of absorption by the tissues. The urinary excretion of vitamin C never becomes equal to the amount ingested, even when the body is apparently completely saturated, owing to destruction of some of the vitamin in the tissues [822]. Half to two-thirds of the ingested vitamin C is metabolized [877] to dehydroascorbic acid, some of which suffers irreversible destruction to diketogulonic acid and oxalic acid [1077]. According to Basu and Ray [322] there is no proportional relationship between the average excretion of vitamin C and the state of saturation, unless the excretion is above 80 mg. or 40 mg. a day.

The average value for the twenty-four hour urinary excretion of vitamin C has been calculated by a number of investigators. Harris [815] and his associates consider that a daily urinary excretion of 18 mg. represents the borderline between a deficient and adequate intake, an excretion of 20 mg. a moderately low intake, and an excretion of 40 mg. a liberal intake of the vitamin. According to van Eekelen [816] a daily excretion of 40 mg. indicates an adequate intake of vitamin C. It is generally assumed that a daily excretion of 25 to 40 mg. represents an average range in most subjects receiving an adequate intake. In the case of infants the daily excretion is under 5 mg. [828]. Small amounts of dehydroascorbic acid are excreted in the urine [1058]. Actually so many factors, including renal

function, affect the excretion of the vitamin, that individual determinations of the vitamin C content of urine are not a reliable index of vitamin C nutrition. The subject will be further discussed under the diagnosis of vitamin C deficiency (p. 506, 548). The factors influencing excretion are considered under the requirements of vitamin C (pp. 522, 528).

The mechanism of the excretion of vitamin C by the kidney has been studied by Ralli [817] and her colleagues. Studies on the simultaneous vitamin C and creatinine urinary clearance in the dog, and vitamin C and inulin clearance in man showed that vitamin C is excreted in the urine by a process of filtration and active tubular reabsorption. This view is supported by the observation of Leblond [818] that the concentration of vitamin C in the capsular fluid of the frog is the same as that of the plasma. The reabsorptive mechanism for vitamin C appears to be limited to a maximal rate, so that when the vitamin is presented to the tubules by the glomerular filtrates at a rate exceeding this maximum, the excess is excreted in the urine. The excretion of vitamin C in a given individual, therefore, depends on (a) the blood plasma level, (b) the rate of glomerular filtration, and (c) the maximum rate of tubular reabsorption. In a later paper Ralli [819] has shown that the vitamin C excretion in the urine does not become zero, even with very low concentrations of vitamin C in the plasma, but ultimately falls to a minimum and constant value and is then independent of the plasma concentration. At lower excretion levels the excretion of vitamin C does not depend on the concentration in the blood nor on the filtration rate through the kidneys, but on the rate of tubular reabsorption, which is extremely variable [817, 819].

Any condition which reduces renal function impairs the excretion of vitamin C and may, therefore, falsify any conclusion regarding the level of vitamin C nutrition when this is based on excretion tests. Sendroy and Miller [820] compared the urinary excretion of patients suffering from renal diseases with that of normal individuals. They found that an abnormally slow excretion of a test dose of vitamin C does not necessarily indicate a low level of the vitamin in the body, because renal damage retards excretion even when the intake of vitamin C is adequate. The effect of decreased kidney function on the vitamin C clearance was found to run parallel to the effect on the urea clearance. Similar findings are reported by Wright and his co-workers [550, 576]. No appreciable destruction of vitamin C occurs in the urine stored in the bladder [821].

Certain foodstuffs appear to increase the amount of vitamin C excreted. Basu and Ray [841] have shown that if cabbage extract is consumed the excretion of vitamin C is temporarily greater than the intake. Musulin [446] and his co-workers have also shown that oats and certain fractions of halibut liver oil cause a marked rise in the excretion. Basu and Ray [841] state that a certain amount of dehydroascorbic acid (p. 446) is found in the urine; the ratio of this to the ascorbic acid lies between 0.05 and 0.4. The excretion of vitamin C is lower in the winter owing to the lower intake during this season [969]. Body posture, rest and exposure to sun have no effect on excretion. Oxygen tension affects excretion of vitamin C. Krasno and his co-workers [974] showed that in man and the guinea-pig moderate degrees of anoxia, corresponding to an

altitude of 18,000 feet for one hour, caused a retention of vitamin C. This effect is probably a metabolic one and appears to influence the utilization or synthesis of vitamin C. The observation is of interest in connection with the statement that vitamin C increases the altitude tolerance of mice [975].

Various drugs affect the excretion of vitamin C. For example, Hawley [824] found that normal persons saturated with vitamin C excreted almost a hundred per cent. of daily test doses when ammonium chloride was given with it. Salicylates, atropine, aspirin, cinchophen, barbiturates, amidopyrine, antipyrine, adrenalin, chloroform, chloretone, paraldehyde, stilbæstrol, œstradiol, and sulphonamides also increase the excretion [825-829]. The commoner anæsthetics increase the urinary excretion and plasma vitamin C [1044]. Insulin and sodium bicarbonate cause a lowered urinary excretion of vitamin C [824, 830]. Exercise and pyrexia decrease the excretion (pp. 521, 522).

Increased and decreased absorption due to drugs or other factors is not necessarily due to altered utilization or urinary excretion of vitamin C. It may be due to changes in renal tubular absorption. For example, œstrogens given to dogs increase excretion by this mechanism [327]. Another possible explanation of the increased secretion of vitamin C after the administration of certain drugs is that body synthesis of vitamin C may be stimulated to detoxify compounds foreign to the tissues.

HUMAN REQUIREMENTS OF VITAMIN C

Methods used for Determining the Human Requirements of Vitamin C.

Dietary Studies. From studies on the amount of vitamin C needed to prevent scurvy the physiologically indispensable minimum requirement of the vitamin has been calculated. Estimates arrived at by independent means have placed the indispensable minimum at between 15 and 30 mg. a day. In 1938 the Technical Commission on Nutrition of the League of Nations adopted the value of 30 mg. "as the minimum for protection against scurvy." Rietschel and Mensching [842] point out that there are several cases on record of healthy persons living on diets containing less than 15 mg. of vitamin C daily for many months without the appearance of scurvy. For example, Nansen, the explorer, lived from August 1895 to June 1896 on meat alone, which probably did not provide more than 10 mg. of vitamin C daily, yet he did not get scurvy. It is also recorded that a Flemish workman suffering from scurvy got better with rest in bed and a diet which only provided 10 mg. of vitamin C daily [351]. He subsequently kept quite well on a diet providing 25 mg. of vitamin C daily. Mensching existed for a hundred days on a diet chiefly of well-cooked meat and cereals, and during this period the blood vitamin C fell from 0.72 to 0.06 mg. per 100 c.c. and the urinary excretion was only 1.0 mg. per 100 c.c. There were no signs of scurvy and the subject apparently felt quite well and gained in weight, although the diet could not have contained more than 10 mg. of vitamin C a day. Pijoan [1075] studied patients receiving only 15 mg. of vitamin C daily, yet they did not develop scurvy.

Rietschel and Mensching suggest that the human requirements of vitamin C are low, probably between 10 and 20 mg. a day, because the vitamin can act catalytically and be alternatively oxidized and reduced. They also believe that synthesis in the human body cannot be excluded.

The possibility that vitamin C may be synthesized in the body has not been taken into consideration in estimating the human requirements of the vitamin. Until recently it was thought that only man, the primates and the guinea-pig suffered from scurvy and that other animals synthesized their vitamin C if necessary. There is now evidence that some of the ungulates (deer, cattle, swine) may also be susceptible [852-854]. The difference between the ability of different species to synthesize vitamin C may be only one of degree. Since many animals are known to synthesize it, it is possible that man can, although not very effectively. Some observers believe that the human foetus is able to synthesize vitamin C because the pregnant woman rarely suffers from scurvy, even on a diet very poor in the vitamin. The fact that a nursing mother may secrete more vitamin C in her milk than she consumes in her food suggests that she can synthesize the vitamin [395]. It is possible that man normally depends on outside sources for his vitamin C, but when these supplies are depleted he synthesizes some of his own.

Stepp and Schröder [346] consider that although approximately 20 mg. of vitamin C a day may prevent human scurvy, such a quantity is not sufficient for perfect health. They state that the dose of vitamin C required to protect guinea-pigs against scurvy does not prevent dental lesions. They also point out that nature gives some indication of the human requirements of vitamin C, since she provides the breast-fed infant of a healthy well-nourished mother with some 40 to 50 mg. of the vitamin a day in its milk. An analysis of the diet of athletes in the Olympic games shows that they were receiving as much as 300 mg. of vitamin C a day. Although this amount may be utilized in severe exercise, there is no proof that such quantities are needed under normal circumstances.

Stefanson [348], who lived in New York for four hundred days on a diet of meat and fat without getting scurvy, stresses the gross discrepancy observed between the laboratory calculations on the requirements of vitamin C, which range from 80 to 100 mg., and the actual amounts obtained by native races who do not get scurvy. The Eskimo, for example, who lives mainly on meat, fish and fat, does not get scurvy, yet his consumption of vitamin C is probably not above 20 mg. a day. Of course, Eskimos may get their vitamin C in special ways, like the North American Indian, who eats the raw adrenals of the moose. Fox [344] and his co-workers state that large burly South African mine labourers remain in perfectly good health on 15 mg. of vitamin C a day. On the other hand, large epidemics of scurvy have occurred in the past in armies in the field and in the Navy on diets that certainly provided no less vitamin C than some native diets. It may be that the vitamin C requirements depend partly on race.

Crandon's observations on experimental scurvy are of considerable

interest [68]. After he had induced clinical scurvy in himself and was excreting no vitamin C in his urine, he found from urinary excretion tests that from 4 to 6 grams were required to resaturate himself. Since the first signs of scurvy appeared after a hundred and thirty-two days of total vitamin C deficiency it was concluded that the maximal daily utilization of the vitamin was between 30 and 45 mg. a day, although it was suggested that the true requirements were below this figure.

Fox [847] kept a healthy native prisoner on a diet free from vegetables, his only source of vitamin C being that present in well-cooked meat. He was under close observation for ten months, during which time he was given active work to do. The vitamin C level in his urine fell to very low levels—sometimes as low as 1 mg. in twenty-four hours—yet he appeared in fairly good health and showed no signs of scurvy, or even increased capillary fragility, nor did he show any dental lesions.

Widdowson and Alington [888] from a survey of middle class diets in 1985 concluded that the average vitamin C consumption was 57 mg. a day. This dropped to 27 mg. in 1941 as a result of the disappearance of fruit and raw salads from the market owing to the war. Actually three-quarters of the persons in the 1941 survey were receiving less than 30 mg. a day without any obvious signs of ill health. Francis and Wormald [868] have also noted that the level of vitamin C nutrition in a group of medical students was not affected during the first year of the war, *i.e.*, up to September, 1940, but that after about twenty-one months, *i.e.*, by July, 1941, the same students showed a considerable drop in their vitamin reserves, as measured by saturation tests. Harris [867] found that the level of vitamin C nutrition, as judged by saturation tests (p. 544), has fallen during the war, particularly in the winter months (1942). This is attributed to the shortage of foodstuffs containing vitamin C. A survey in an English public school showed that the daily intake was only 15 mg. [971]. The average daily intake in the British Army is less than 25 mg. [972]. In the R.A.F., which is considered to be a very fit body of men, the daily intake of vitamin C varies from an average of 17 mg. in March to 26 mg. in October and November [1042]. Ungley [978] observed no evidence of scurvy in Navy personnel receiving 16 to 30 mg. of vitamin C daily; this was mainly in potatoes. Harris [867] has observed a seasonal tide in the vitamin C levels; low after the winter months, when salads and greenstuffs are scarce, and better after the summer months, when they are more plentiful.

Determination of Capillary Fragility. According to Göthlin [848] the capillary fragility test (p. 589) serves as a measure of the physiological indispensable minimum requirement of vitamin C, since it can be used to determine the quantity of vitamin C necessary to maintain normal capillary fragility. From simultaneous determinations of capillary fragility tests and blood ascorbic acid levels Göthlin and others state that the capillary fragility becomes pathological at blood values between 0.1 and 0.14 mg. of vitamin C per 100 c.c., which is considerably below the figure of 0.6 mg. per 100 c.c. calculated by other methods. From capillary fragility tests Göthlin puts the minimum daily requirements of vitamin C at 20 to 30 mg. for a 60 kg. person. He studied four schizo-

phrenics over a period of six to eight months. They were first placed on diets low in vitamin C and the amount of the vitamin in the food increased at regular intervals until their capillary resistance, which was low at first, was restored to normal levels. From 0.39 to 0.48 mg. of vitamin C a day per kilo of body weight was necessary to achieve this. The corresponding figures for a person weighing 60 kg. are approximately 23 to 29 mg. a day. Earlier estimates carried out in 1931 with orange juice gave figures varying from 21 to 30 mg. a day. More recent work suggests that the capillary fragility test is of no value in detecting vitamin C deficiency (p. 539).

Urinary Excretion of Vitamin C. Harris and his associates [355, 356] arbitrarily accepted the figure of 25 mg. a day as the "minimal-optimal" requirement of vitamin C, basing this on the work of Göthlin (p. 505) and on the amount of vitamin C in a ration of lemon juice that just protected against scurvy. They found that adult subjects on a daily intake of 25 mg. of vitamin C excreted about 18 mg. daily in the urine. They concluded that if a subject excretes less than 18 mg. of vitamin C a day and fails to respond by a marked increase in excretion on the first or second day after the administration of a test dose of 700 mg. per 10 stone of body weight, the diet contains less than the reputed minimum-optimal quantity of vitamin C. Daily excretion values of 18 mg. were taken to represent the borderline between adequacy and deficiency; a daily excretion of 20 mg. a moderately low intake; and a daily excretion of 40 mg. a liberal intake. According to Spellberg and Keeton [357] the minimum normal daily excretion is generally considered to be about 20 mg. van Eekelen [358] considers a daily excretion of 40 mg. an indication of an adequate intake of vitamin C. It is now questioned whether the amount of vitamin C normally excreted in the urine can be used to determine the adequacy of the intake (p. 543). Deeny [970] has shown that marked hourly variations occur in the excretion of vitamin C, which is present at all physiological blood levels of the vitamin and is not related to the pH of the urine or to the rate of the flow. These hourly variations throw some doubt on the validity of excretion studies for the detection of vitamin C deficiency (see p. 543). Because of the variable factors in the excretion of vitamin C, "saturation" tests (pp. 544-549) have been introduced for the assessment of vitamin C deficiency.

The rationale of saturation tests depends on the hypothesis that after the administration of a test dose of vitamin C the requirements of the tissues must be first satisfied before the concentration of the vitamin in the blood rises to and is maintained at a sufficiently high level for it to be excreted in the urine. Briefly the method consists of determining the daily excretion during a period in which large doses of vitamin C are given to saturate the tissues. It is discussed in detail later (p. 544).

van Eekelen [363-365] estimates the human requirements of vitamin C by saturating the individual with a daily dose of 250 mg. of vitamin C, then depleting the reserves by giving a diet as deficient as possible in vitamin C for a period of several weeks, and then saturating again with 250 mg. a day. The number of days that elapse before a marked increase in excretion occurs is noted in each case. The total intake of vitamin C

during the second saturation period divided by the number of days needed to reach saturation is considered to be the daily requirement of vitamin C. For an adult of 70 kg. this is 60 mg. a day. This may be considered to be optimal since the patient is saturated. There is no evidence that saturation with vitamin C is essential for good health, although several authors believe so. The urinary excretion of vitamin C following test doses may also be abnormal in patients with impaired renal function (p. 502). Göthlin [866] has criticized this method because the results will depend upon the period during which the subject has been deprived of vitamin C.

van Eekelen believes that the daily requirement of vitamin C depends on the existing storage of the vitamin. If the diet is normally low in vitamin C, or if there has been a prolonged period of depletion the daily requirement is lower than if the diet has been rich in the vitamin or if the depletion period is short. Thus the requirements or "daily use" of vitamin C amounted to 34 mg. in a subject after a depletion period of ninety-four days and to 63 mg. after a period of only twenty-seven days.

Using a saturation method Heinemann [868] arrived at the figure of 0.8 mg. of vitamin C per day per kilogram of body weight, which corresponds to 56 mg. a day for a person weighing 70 kg. Armentano [480] gives a similar figure. Heinemann considers this to be an optimum requirement; a daily intake of 0.4 mg. of vitamin C per kilogram of body weight, or 28 mg. a day, protects against scurvy. Schultzer [869] saturated a scorbutic patient weighing 63 kg. in four weeks with a daily dose of 40 mg. of vitamin C given intravenously. There is, however, some evidence that an intravenous test dose of vitamin C, especially if large, results in a sudden rise in the plasma concentration, and a transitory overflow into the urine, even though the tissues are not saturated [870].

Kellie and Zilva [871] carried out saturation tests to determine the daily requirements of vitamin C. On a daily dose of 30 mg. of vitamin C the appearance of the latter in the urine almost ceased. On the other hand, on a dose of 50 mg. twenty to thirty per cent. of the intake was excreted at equilibrium when descending from a higher dose and eight to ten per cent. when ascending from "unsaturation." From this it was assumed that the minimum dose necessary to bring about saturation was between 30 and 50 mg. a day. Kellie and Zilva point out that this by no means represents the minimum daily dose necessary to prevent the onset of scurvy or even to maintain good health. In studies on guinea-pigs [872] they found that although 20 mg. of vitamin C a day was necessary to "saturate" a guinea-pig, a quarter of this dose protected the animal against scurvy. It was, therefore, concluded that about 15 mg. of vitamin C a day would be sufficient to prevent scurvy and maintain an individual in good health. In certain circumstances the food of apparently healthy individuals does not contain much more vitamin C than this. But even if such quantities may be sufficient under optimal conditions, it would seem desirable to supply more than this as a factor of safety against altered circumstances which may increase requirements (pregnancy, infection, heavy work, etc.).

A somewhat different procedure has been worked out by Hauck [874] and her associates. The individual is first saturated with vitamin C by the daily administration of 200 mg. for six days; that saturation is complete is confirmed by an increase in excretion following a single test dose of 400 mg. The test is then repeated two or three times to establish the individual response to the 400 mg. dose when saturation is complete. Finally a series of similar tests is conducted on graded doses of vitamin C and the smallest amount found that will induce a similar response to the test dose of 400 mg. as obtained in the preliminary tests. The values obtained represent maximum requirements. Hauck found that 1 to 1.6 mg. of vitamin C a day per kilogram of body weight are necessary to maintain tissue saturation. This corresponds to 65 to 100 mg. daily for a person weighing 10 stone. Of the seven subjects studied by Hauck two required 70 to 85 mg. a day; three required 85 to 100 mg.; and two required more than 100 mg.

These findings were confirmed by Todhunter and Robbins [875]. A basal diet furnishing 20 mg. of vitamin C a day was supplied to three volunteers, and the response in urinary excretion to a 400 mg. test dose of vitamin C following a four-day period when 200 mg. daily was taken, was determined for three successive periods. It was then found that an intake of 1.6 to 1.7 mg. of vitamin C per kilo of body weight was necessary to give a similar response to the test doses. This corresponds to about 100 mg. a day for a person of average weight. Blood values were also calculated and it was found that at an intake of 60 mg. a day the plasma vitamin C was above 1 mg. per 100 c.c.; more than 120 mg. a day was necessary to raise the blood plasma level to 1.4 mg.

Another technique has been used by Widenbauer [876], who keeps the subject under test on a uniform diet containing very little vitamin C for several days. The vitamin C content of the urine is determined on two twelve-hour specimens and the average daily excretion calculated. This is the preliminary blank reading. Test doses of from 200 to 500 mg. of vitamin C a day are then given until saturation is reached, as known by an excretion of at least fifty per cent. of the last test dose in twenty-four hours, or half of this dose in twelve hours (p. 545). The daily dose is then adjusted to give an excretion of vitamin C slightly higher than the preliminary blank reading. When this dose has been determined it is given over a final period of seven days, the urine being titrated daily to find the average excretion. The daily requirement is then found by taking from the average daily intake during the final period the average daily excretion during this period less the average daily excretion of the preliminary period. According to Widenbauer the daily requirements of vitamin C vary from a minimum of 30 mg. to an optimum of 50 mg. a day. Wachholder [877] gives similar figures. Lewis and her co-workers [976], using a similar method, state that adults receiving 75 mg. of vitamin C daily maintain tissue saturation. This figure is taken to be the daily requirement of the adult.

It must be stressed that the human requirements of vitamin C based on excretion and saturation tests are purely arbitrary figures. It has not been proven that an individual in a state of saturation is any healthier

than one who is not. In fact many people apparently in the best of health are "unsaturated" and excrete very little vitamin C. The value of the test dose technique for performing saturation tests is also open to grave criticism. Both the size of the test dose and the criterion of adequate excretion vary from one worker to another.

Concentration of Vitamin C in the Blood. Several methods have been devised for estimating the vitamin C of the blood (p. 549). The range of blood plasma values encountered even amongst those regarded as normal is a wide one, however. Many observers believe that plasma vitamin C values below 0.5 to 0.7 mg. per cent. indicate a state of vitamin C depletion and that values of 0.7 to 1.0 mg. indicate a mild deficiency. For normal well-fed people 1.2 to 1.5 mg. per cent. is a common range of concentration; Ralli [878] and her associates obtained the value of 1.2 mg. for a group of students. Isolated blood plasma vitamin C values are of little diagnostic use unless they are very low. Even so, blood levels of 0.02 mg. per 100 c.c. have been reported in the absence of scurvy [867]. According to Wright [404] 25 mg. of vitamin C a day is sufficient to maintain whole blood or plasma values when human subjects are on a vitamin C free diet.

Bessey, White [858] and Horwitt [859] have determined the vitamin C requirements by observing the amount of vitamin C sufficient to produce an optimal post-absorptive level of the vitamin in the blood plasma. The amount of vitamin C is found by giving supplements in increasing amounts until there is a gradual rise in the plasma vitamin C. The value is about 50 mg. daily. Todhunter, Robbins and McIntosh [861] have also observed that the plasma vitamin C begins to rise in normal subjects after the ingestion of 50 mg. of vitamin C, either as the synthetic compound or from fruits.

Fincke and Landquist [977] consider 0.8 mg. of vitamin C per 100 c.c. of blood as an index of adequate nutrition, and they find that a daily dose of 40 to 90 mg. daily, or 0.8 to 1.2 mg. per kilogram of body weight is needed to secure this. To maintain a level of 1.0 to 1.2 mg. per 100 c.c. of blood, which is considered optimal by some workers [878], a daily intake of 110 to 180 mg. was found necessary.

In view of the fact that very low levels of blood ascorbic acid have been recorded in the absence of scurvy or apparent ill-health, vitamin C requirements based on blood levels must be accepted with considerable caution (*cf.* p. 549).

Correlation of Vitamin C content of Blood and Urine. According to Faulkner and Taylor [880] blood vitamin C values above 1.4 mg. per cent., the threshold value, correspond to a state of vitamin C saturation. while lower values correspond to an "unsaturated" condition. There is some evidence that the threshold value varies in different individuals, who, therefore, exhibit a wide range in "saturation" capacity [881, 882]. This makes the test valueless.

Ralli and her co-workers have argued that the amount of vitamin C required daily would be the smallest amount necessary to maintain a normal plasma level. At this level the amount excreted should be small and should remain fairly constant so that the maximal reabsorptive

capacity of the kidney tubules (p. 502) would be exceeded. If more than the daily requirement is given the excretion should rise promptly.

In the technique used by Ralli [878] for estimating the daily requirements the test subjects are hospitalized and given diets containing not more than 5 mg. of vitamin C a day. The daily (twenty-four hour) urinary excretion of vitamin C is determined and also the vitamin C in the blood plasma. On intakes of 50 mg. over a period of a hundred and twenty-seven days the average plasma vitamin C was 0.4 mg. per 100 c.c. The intake of vitamin C was increased, but as long as it did not exceed 100 mg. the excretion remained constant and rarely exceeded 15 mg. in twenty-four hours, although the plasma level increased. With an intake of more than 100 mg. of vitamin C a day, a sharp rise in the urinary excretion occurred and a plasma concentration of 1.0 mg. per cent. was maintained. This is taken as the saturation level of the blood. Ralli, therefore, concludes that to maintain tissue saturation and a plasma level of 1.0 mg. per cent. at least 100 mg. of vitamin C must be consumed daily; this is an optimum intake. With a daily intake of 50 mg., although the plasma concentration only averaged 0.4 mg. per cent., there were no symptoms of vitamin C deficiency, and it was suggested that this value represents the lower limit of normal. With a daily intake of 75 mg. the plasma value could not be raised to or maintained at 1.0 mg. per cent. Chinese workers also put the maximal requirements of vitamin C at about 100 mg. a day [888]. Ralli considers that in fully grown adults the daily vitamin C requirement is not dependent on age, height or weight.

Goldsmith [551] and her associates saturated twelve patients with 100 mg. of vitamin C three times a day for a week, then twice a day for a second week, and then a maintenance dose of 50 mg. a day was given. At the end of fifteen to eighteen weeks all the patients showed blood vitamin C levels of more than 1 mg. per 100 c.c., which was considered to indicate saturation with the vitamin. Taking into consideration the fact that the patients were receiving 20 mg. of vitamin C daily in their diet as well as a supplement of 50 mg. the daily requirements were estimated at 70 mg.

Purinton and Schuck [988] consider that a plasma level of 0.8 mg. or more per 100 c.c. indicates an adequate level of vitamin C nutrition. To determine the human requirements of vitamin C they measured the vitamin C blood concentration and urinary elimination of sixty-three healthy individuals in response to a test dose of 500 mg. The subjects were given a diet containing 14 to 15 mg. of vitamin C and fasting urine and blood levels determined. Then the test dose was given orally or intravenously and the plasma vitamin C measured at regular intervals until the absorption of the dose was indicated by a return of the plasma value to the fasting level. The urine was also analysed at definite intervals for twenty-four hours following the test dose. By subtracting the amount of vitamin C excreted in the test period from the quantity administered in the diet and the test dose a retention figure was obtained. By subtracting the quantity of vitamin C excreted under fasting conditions from this retention figure a value was obtained which was considered to be the quantity of vitamin C metabolized by the individual. Using this method,

the vitamin C requirements of the young adult are of the order of 100 mg. daily.

These tests are based on arbitrary blood levels and the concept of saturation, which as explained elsewhere (p. 549) is not a normal condition, but a definitely abnormal one since most individuals are not "saturated" on an average diet. Either supplements of synthetic vitamin C must be taken or a large quantity of fruit or vegetables must be consumed. There is no clinical justification for the view that a level above 0.5 mg. to 0.7 mg. per 100 c.c. of blood is necessary for optimal health.

REQUIREMENTS UNDER VARIOUS CONDITIONS

Infancy. The foetus withdraws vitamin C from the mother through the placenta, and the concentration in the blood plasma of the umbilical cord has been found by a number of investigators to be from one and a half to four or more times that present in the maternal blood at the time of delivery [388-392]. In a group of twenty-two hospital cases Braestrup [389] obtained average values of 0.26 mg. of vitamin C per 100 c.c. of maternal blood and 1.07 mg. for the blood value of the umbilical cord. The capillary blood value of the infant was 0.69 mg. per 100 c.c., and this dropped to 0.27 mg. ten days after birth. Lund and Kimble [979] give the average vitamin C level in maternal blood at the time of delivery as 0.68 mg. per 100 c.c. and in the foetal blood as 1.32 mg. McDevitt and co-workers [978] have presented proof that the placenta exerts a selective action in filtering vitamin C from the maternal blood. The plasma vitamin C of the infant varies considerably during the first two weeks of life and depends upon the amount of vitamin C in the diet, showing that the infant has little or no capacity for storing the vitamin. It would appear that the foetus withdraws considerable amounts of vitamin C from the mother during pregnancy and stores it, since the maternal plasma vitamin C drops considerably towards the end of pregnancy, being at delivery less than one-half of its value during the first twenty-eight weeks of pregnancy [391]. This is also confirmed from a study of the livers of infants coming to autopsy. The concentration of vitamin C in the liver is high at birth, but in infants a month old the liver reserves have fallen considerably, and from that age the concentration depends on the amount of vitamin C in the diet [393]. These figures clearly indicate the need for an increased intake of vitamin C in pregnancy.

The rapid decrease in the vitamin C content of the blood during the first few weeks of life suggests that the reserves at birth are soon exhausted and that vitamin C must be regularly supplied, either through breast milk or by supplements of fruit or vegetable juices to maintain a normal blood concentration. Mindlin [394] states that two weeks after birth the average plasma vitamin C is 0.1 mg. per 100 c.c. compared with 0.4 to 0.8 mg. in the case of slightly older infants receiving supplements of 20 mg. of vitamin C. Braestrup [389] also observed that low blood levels in infants can be raised to from 0.56 to 0.76 mg. per 100 c.c. by the administration of supplements of 20 mg. of vitamin C a day, although 10 mg. had little effect. He suggests that in the early weeks of life not

less than 20 mg. of vitamin C is needed a day, or approximately 5 to 6 mg. per kilogram of body weight.

The unreliability of calculations based on plasma vitamin C levels alone is pointed out by Snelling [401], who found low plasma values in some infants without any signs of scurvy. Taking plasma vitamin C levels as a criterion of vitamin C nutrition Bakwin [402] showed that an artificially fed infant requires a daily supplement of 75 to 100 mg. to raise its plasma vitamin C to the same level as that of a breast-fed infant of a well-nourished mother.

The vitamin C content of the food of the breast-fed infant depends on the mother's intake of the vitamin. Human milk has been found to contain from 1 to 10 mg. of vitamin C per 100 c.c. Ingalls, Draper and Teel [895] have shown that during the first two weeks of lactation fresh breast milk from mothers on the usual diet of a maternity ward contains an average of 4.5 mg. of vitamin C per 100 c.c.; the breast-fed babies of such mothers, therefore, receive an average of 28 mg. of vitamin C daily, assuming an intake of 680 c.c. of breast milk a day. When the mother receives a liberal diet containing plenty of vitamin C, the content of the breast milk is increased to 7.8 mg.; the infants of such mothers then receive nearly 50 mg. of vitamin C in their milk. Ingalls and his co-workers [895] have shown that from 20 to 50 mg. of vitamin C a day are provided by the breast milk of mothers receiving less than 20 mg. of vitamin C a day. Either the mother's tissue reserves are drawn upon or possibly synthesis of vitamin C occurs. These findings show that the vitamin C requirements are considerably increased in the lactation period. It is considered that levels of less than 4.0 mg. of vitamin C per 100 c.c. of milk are inadequate [896-898]. 4.0 mg. per 100 c.c. of milk would supply 20 to 40 mg. of vitamin C a day to the infant, assuming that 500 to 1,000 c.c. of milk are consumed a day. Baumann and Rappolt [898] consider 8 mg. per 100 c.c. to be the maximum possible value for breast milk.

Dann [871] suggests that the vitamin C requirement of premature infants is related to protein intake, since after a period of saturation with vitamin C breast-fed infants retain a larger part of a "test dose" (p. 873) of the vitamin in their tissues than do infants fed on cow's milk. This means that the vitamin C requirement of the artificially fed infant is greater than that of the breast-fed, and a possible explanation of this may be found in the difference in the protein content of human and cow's milk. Human milk contains about 1.5 gm. of protein per 100 c.c. and provides the premature infant with 2.6 to 2.8 gm. per kg. daily, while cows' milk provides from 4.6 to 6.1 gm. per kg. daily. This hypothesis, that an increased requirement for vitamin C is related to a higher level of protein intake, receives support from the observations on vitamin C and amino-acid metabolism (p. 497). Observations by Patterson and Bourquin [980] suggest that a high protein diet increases the requirements of vitamin C.

It was formerly assumed that vitamin C need not be given to infants under three months of age. The Technical Committee on Nutrition of the Health Organization of the League of Nations (1988) recommend the daily addition of 10 to 30 c.c. of orange juice (equivalent to 5 to 15 mg. of

vitamin C) to the feeds of artificially fed infants "as from the age of three months." If an infant is breast fed or the mother is not adequately nourished it needs supplements of vitamin C, preferably in the form of diluted vegetable and fruit juices, which should be given before it is three months old.

The plasma of artificially fed infants a few weeks old not receiving additional vitamin C was found by Snelling [401] to contain between 0.05 mg. and 0.52 mg. of vitamin C per 100 c.c., which is considerably below normal levels. Ingalls [899] also describes three cases of premature infants fed on pasteurized milk to which no vitamin C was added, all of whom died in thirty-two to fifty-seven days with post-mortem evidence of scurvy. A scorbutic dietary of three months would exhaust the vitamin C stores of the infant. Pasteurized milk may contain as little as 0.8 mg. of vitamin C per 100 c.c., so that infants receiving this will only get 1.5 to 3 mg. of vitamin C a day; this is not enough to prevent scurvy. Vitamin C should, therefore, be given in the first few days of life to premature and artificially fed infants in quantities equivalent to those in breast milk (20 to 40 mg. a day). If the mother's breast milk provides less than 4 mg. per 100 c.c. vitamin C supplements should also be given to the infant.

Neuweiler [400] calculated the requirements of very young infants of nine to twelve days by a test dose technique. He assumed that infants with a satisfactory level of vitamin C nutrition should excrete within twenty-four hours more than fifty per cent. of test doses of 80 mg. of vitamin C given subcutaneously. These figures were evidently arrived at from a test dose of 600 mg. for an adult of 10 stone. Neuweiler concluded that the daily vitamin C requirements of the very young infant are in the region of 6 mg. per kilogram of body weight, or 20 to 25 mg. a day for babies of average weight shortly after birth.

How much vitamin C the infant needs for optimal nutrition is still a matter for dispute. The absolute minimum required to protect infants under one year of age against scurvy is 10 mg. a day according to Macy and co-workers [408], who have made a clinical examination of four hundred and twenty-seven infants. From analogy with guinea-pig studies at least twice this amount would be required for normal tooth development, and double this is necessary for adequacy, making 40 mg. daily the requirement in infancy. Szent-Györgyi showed that although 1.5 to 2 mg. of vitamin C a day will protect a guinea-pig against clinical scurvy, it requires a much higher dose to keep the animal in good health and free from infection. In its natural environment it may receive as much as 20 to 40 mg. of vitamin C a day. It is reasonable to assume that the physiological requirements of an organism for a particular vitamin are satisfied by the diet provided in its normal environment. In the case of the infant this may be taken to mean that his vitamin C requirements are satisfied by the breast milk of a healthy mother. Obviously the vitamin C content of breast milk depends on the diet of the mother, and this is very variable, even in times of plenty.

Human breast milk contains from two to six times as much vitamin C as cow's milk [810]. As shown by titration with 2:6-dichlorophenol-

indophenol the breast milk of healthy women contains from 4 to 8 mg. of vitamin C per 100 c.c. of milk during the early months of lactation [405-409]. Levels below 4 mg. have been considered a sign of vitamin C deficiency on the part of the mother [898], although a group of over four hundred nursing mothers in Gothenberg showed values averaging 8.9 mg. [808]. Assuming an intake of 21 ounces of milk a day in early infancy a daily intake of 40 to 50 mg. of vitamin C is quite possible according to the estimates of Selleg and King [409]. Widenbauer [410] has reported values of from 18 to 78 mg. of vitamin C in a day's supply of milk from wet nurses, using the test dose technique; the higher figure is, of course, not representative of the ordinary figures for vitamin C in breast milk. Neuweiler [400] considers that an infant gets 25 mg. of vitamin C through its milk in the early days of life and about 50 mg. at six months. Ingalls [899], on the basis of 4 mg. of vitamin C per 100 c.c. of milk, estimates that the breast-fed child receives from 20 to 40 mg. of vitamin C a day, assuming that its fluid intake is 500 to 1,000 c.c. daily. Braestrup [889] calculates that the breast-fed infant with an intake of 150 c.c. of milk a day per kilo of body weight receives from 4 mg. to 8 mg. of vitamin C per kilo of body weight in its first few months of life. This corresponds to an intake of 15 to 40 mg. a day.

The vitamin C requirements of infants advocated by the authorities quoted fall between the values of 20 to 50 mg. a day or from 5 to 8 mg. per kilo of body weight. These quantities can be satisfied by a nursing mother on a liberal diet, but they are not received by artificially fed infants unless they receive supplements of fruit juice or the synthetic vitamin. The vitamin C content of cows' milk varies from 0.7 to 2.9 mg. per 100 c.c. of raw milk and from 0.3 to 2 mg. when pasteurized [411, 412]. Thus raw milk would provide from 3.5 to 29 mg. of vitamin C a day (assuming a daily intake of 500 to 1,000 c.c. of milk) and pasteurized milk from 1.5 to 20 mg. By the time the cows' milk reaches the baby its vitamin C content is much lower still, particularly if it is boiled before use. Hawley [413] has shown that a typical sample of raw milk delivered to a hospital contained 1.65 mg. per 100 c.c.; after pasteurizing it dropped to 0.9 mg., and after preparation for consumption it contained only 0.61 mg. She estimates that cows' milk after preparation only supplies the human infant with from 4 to 6 mg. of vitamin C a day, if it consumes 600 to 700 c.c. of milk. Assuming an optimum requirement of 30 to 50 mg. of vitamin C a day, and that cows' milk provides a maximum of 10 mg. of vitamin C, Hawley considers that the bottle-fed infant needs a supplement of 20 to 40 mg. of vitamin C a day. Under normal conditions the vitamin C can be given as diluted orange, fruit or vegetable juice. Natural sources supplying approximately 20 mg. of vitamin C a day are:—

15 c.c. or $\frac{1}{2}$ ounce (= 1 tablespoonful)	black currant juice.
30 c.c. or 1 ounce	black currant purée.
50 c.c. or $1\frac{1}{2}$ ounces	red currant juice.
50 c.c. or $1\frac{1}{2}$ ounces	orange juice.
50 c.c. or $1\frac{1}{2}$ ounces	grape fruit juice.
50 c.c. or $1\frac{1}{2}$ ounces	lemon juice.
10 c.c. or $\frac{1}{2}$ ounce	rose hip tea or syrup.

20 c.c. or $\frac{3}{4}$ ounce	.	.	.	rose hip jam or jelly.
70 c.c. or 2 $\frac{1}{2}$ ounces	.	.	.	cabbage water.
100 c.c. or 3 $\frac{1}{2}$ ounces	.	.	.	spinach water
100 c.c. or 3 $\frac{1}{2}$ ounces	.	.	.	turnip or swede juice.
100 c.c. or 3 $\frac{1}{2}$ ounces	.	.	.	tomato juice.

These should be freshly prepared for each feed, or at any rate twice daily. There is no need to give synthetic vitamin C when the cooking water from "greens" is available.

Requirements of Children. Harris and Ray [406] concluded that the vitamin C requirements of children are greater than those of an adult calculated per unit of body weight. This conclusion was supported by the work of Everson and Daniels [415], who studied three boys aged thirty-nine, fifty-seven and fifty-nine months. These children were kept under careful observation and given graded diets containing known and increasing amounts of vitamin C over five-day periods. With increasing intakes of vitamin C the retention (intake less output) of the vitamin kept pace with the intake up to a certain point, beyond which increased intake did not result in increased retention. The peak of retention occurred at intakes of 7.5, 6.6, and 6.4 mg. per kilo of body weight in the three children; higher levels of ingestion caused no increase in retention. Using "saturation" as a criterion of an adequate intake of vitamin C, or an excretion exceeding fifty per cent. of the intake (p. 546), the average daily intakes of the three children were 117, 121 and 120 mg. respectively.

Widenbauer [376] obtained much lower figures in his studies on a boy of two to three years of age. The child was kept on a diet low in vitamin C for a few days and then given oral test doses of the vitamin until he was saturated, as shown by an excretion of at least fifty per cent. of a half-day test dose in twelve hours (p. 546). The method gives the minimum vitamin C requirements, which in this case amounted to 21 to 22 mg. daily; the requirements of an adult measured by the same technique were between 26 and 28 mg. If these figures are accepted the requirements of the child must be regarded as being only slightly less than those of the adult, and cannot be calculated in terms of body weight as suggested by Harris and Ray. Hathaway and Meyer [886] also state that the vitamin C requirements of children from four and a half to six were not related to body weight, nor were they to age and sex. A daily intake of about 80 mg. of vitamin C was the marginal level to ensure tissue saturation in the children, although the actual "utilization" of vitamin C was 28 ± 2 mg. daily. In a later study they state that 25 mg. of vitamin is sufficient to maintain saturation in young children, provided it is given as orange juice or if potassium citrate is added to the diet [1070]. This appears to increase the utilization of vitamin C.

Bessey and White [858] determined the vitamin C requirements of a group of ninety-three children aged five to thirteen, by finding the amount of orange juice necessary to produce a gradual increase in the plasma vitamin C. This was about 8 ozs. daily, which corresponds to 40 to 50 mg. of vitamin C a day.

A study of the vitamin C requirements of school children aged six to

twelve years has been made by Roberts and co-workers [981]. On the basis of blood and urinary excretion studies they found that a daily intake of 65 to 75 mg. of vitamin C was needed for saturation in school children of this age group. The excretion of fifty per cent. of a test dose of 800 mg. in twenty-four hours (p. 546) and a blood level of over 0.7 per cent. were taken as criteria of saturation. In order to reach threshold values (about 1.4 mg. vitamin C per 100 c.c. blood), these children required 105 to 125 mg. of vitamin C daily.

Analyses of children's meals have been made to determine the vitamin content and so to compute the daily intake. It does not follow that the vitamin C intake of a group of children taken at random is the optimum. Most nutrition surveys reveal that the vitamin C intake is considerably below that postulated by laboratory studies as the optimum. James [982] has shown that some Hertfordshire school children have a daily intake of only 10 to 15 mg. of vitamin C, some even falling below 10 mg. Macdonald [984] of Leicester determined the vitamin C content of school lunches, which provide the bulk of the day's intake of vitamin C, and found that it varied from 4.2 to 28.3 mg. Assuming breakfast and tea contained a little vitamin C, the daily intake of those receiving the lower figure could not be more than 10 mg. daily, while that of those on the higher figure could not be more than 35 mg. Marrack and his colleagues [984] from analyses of meals in British Restaurants and school canteens, showed that unless green vegetables are included in the menu, the daily intake of vitamin C may be only about 15 mg. At two public schools Widdowson and McCance [996] found that before the war the daily intake of vitamin C was 33 mg.; this had fallen to between 15 and 19 mg. daily in 1942. In one of the schools neither green vegetables nor salad appeared on the menu. The intake at a day school was better—32 mg. daily—because green vegetables were eaten. The experience of Harris and Olliver [993] was a little better. They carried out an all-the-year round investigation of the diet in a residential children's home and found that the lowest intake in spring and winter was 19 to 24 mg. daily, and the highest in summer and autumn was 24 to 55 mg. daily. In this home dietary practice was particularly good, and included fresh green vegetables and potatoes. Harris states that the level of vitamin C nutrition in poor working-class homes is particularly bad. Payne [983] carried out saturation tests on a group of English school children and stated that eighty-six per cent. were getting enough vitamin C.

A considerable discrepancy appears between the optimum vitamin C intake calculated from laboratory tests and the actual intake found in practice. One is tempted to ask whether an intake of more than 10 to 15 mg. daily is really necessary. Are levels of this order detrimental to health? No gross signs of disease that can be attributed to vitamin C deficiency have been observed in children at such levels. Trials have shown, however, that some groups of children in apparently normal health benefited from an increased allowance of vitamin C, their average physique or resistance to infection, for example, being significantly better than those of controls on "ordinary" diets [816, 985].

The Requirements of Adults. The calculated vitamin C requirements

of the adult-vary widely with the method used for estimation. The following table gives an analysis of the results obtained.

Author.	Method.	Minimum Daily Requirements in mg.	Optimum Daily Requirements in mg.
Fox [844]	Dietary studies	15	—
Rietschel <i>et al.</i> [842]	"	10-20	10-20 ?
Stefansson [843]	"	20	20 ?
Crandon [68]	"	?	30-45
Göthlin [848]	Capillary fragility	20-30	—
Harris [855, 856]	Urinary excretion	25	—
van Eekelen <i>et al.</i> [868-865]	Saturation tests	—	60
Heinemann [868]	"	28	56
Schultzer [869]	"	28	40
Kellie and Zilva [871]	"	15	30-50
Hauck <i>et al.</i> [874]	"	—	65-150
Levcowich and Batchelder [877]	"	—	50
Ludden <i>et al.</i> [882]	"	—	75-100
Goldsmith <i>et al.</i> [551]	"	—	70
Todhunter and Robbins [875]	"	—	100
Widenbauer [876]	"	30	50
Ralli <i>et al.</i> [878]	Blood and excretion levels.	50	100
Prunty and Vass [1005]	"	—	70
Lewis <i>et al.</i> [976]	"	—	75
Purinton and Schuck [988]	"	—	100
Horwitt [859]	Blood levels	50	—
Kyhos <i>et al.</i> [987]	"	—	50
Dodds and MacLeod [997]	"	35	70 mg. or 1 mg per kilo body weight.
Fincke and Landquist [977]	"	—	40-90
Technical Commission on Nutrition of League of Nations, 1939	—	30	—

It will be seen that the calculated minimum requirements of vitamin C for the adult thus vary from 10 to 50 mg. a day, while the optimum values are from 30 to 100 mg. The higher figures are based on saturation tests. The condition of being "saturated" with respect to vitamin C cannot be regarded as normal and it is not reasonable to suggest that persons not saturated are suffering from vitamin C deficiency.

A study of the diets of adults shows that very few individuals receive the intakes suggested by laboratory tests. The following are some representative values :—

In Stiebeling's reports on American families seventy-two to ninety-six per cent. of diets, costing from \$1.25 to \$1.87 weekly, contained less than the reputed optimum of 75 mg. vitamin C. With a rise of income the intake went up.

Author.	Subjects.	Approximate Daily Intake.
Marrack <i>et al.</i> [984]	Adults	15 mg.
Stuhl [986]	Soldiers	Less than 25 mg.
Widdowson and Alington [888]	Middle class	27 mg.
Widdowson and McCance [996]	Public schoolboys.	15-19 mg.
McNee and Reed [971]	Engineers	Less than 30 mg.
Ungley [973]	Naval ratings and civilians.	16-30 mg. (22.6 mg. per 10 stone)
Fox [844]	African labourers.	15 mg.
Rietschel <i>et al.</i> [842]	—	10-20 mg.
Stefansson [243]	—	20 mg.

Recommended Daily Allowances of National Research Council, U.S.A.

In 1941 the Committee on Food and Nutrition of the National Research Council drew up a table of the probable daily requirements of various vitamins. These were tentative allowances for use in planning practical dietaries. The values recommended for vitamin C are given below :—

Recommended Daily Allowance for Vitamin C National Research Council, U.S.A.

	Calories.	Vitamin C, mg.
Man (70 kg.).		
Moderately active	3,000	75
Woman (50 kg.).		
Moderately active	2,500	70
Pregnancy (latter half) . . .	2,500	100
Lactation	3,000	150
Children up to 12 years.		
Under 1 year	100 per kg.	30
1- 8 years	1,200	35
4- 6 „	1,600	50
7- 9 „	2,000	60
10-12 „	2,500	75
Children over 12 years.		
Girls, 13-15 years	2,800	80
16-20 „	2,400	80
Boys, 13-15 „	3,200	90
16-20 „	3,800	100

It is unwise to rely on a minimal intake of vitamin C, because it does not provide a safety margin for increased requirements in cases of severe exercise, infection or pregnancy. Animal experiments also suggest that the scurvy preventing level of vitamin C does not prevent minor lesions associated with vitamin C deficiency. Thus experiments on guinea-pigs show that whereas 1 mg. of vitamin C daily prevents the onset of obvious signs of scurvy, about 2 mg. is needed for normal tooth development, and 3 mg. or more to protect the animal from injury due to diphtheria toxin.

Requirements and Age. The requirements of infants and children have been discussed. Purinton and Schuck [988] consider that young adults, *e.g.*, aged fifteen to twenty years require more vitamin C than older adults of twenty-five to fifty years. For the young adult they consider 100 mg. is the optimum; for the older adult 80 mg. They have shown that the requirement is influenced by the basal metabolic rate, and this would account for the difference in the two age groups. This would appear to negate the observation of Gander and Niederberger [878] that the vitamin C requirements are increased in old age. This apparent increase may not have been due to increased basal requirements, but to the presence of mild disease and infection which are common in elderly hospital patients, who were the subjects of study. Rafsky and Newman [688] report an increased vitamin C requirement in a group of aged persons (ages sixty-six to eighty-three) who were not suffering from any disease that could be detected clinically. They state that there was increased retention of vitamin C on test dosing. This, however, may have been due to increased retention resulting from a low level of vitamin C nutrition, rather than to increased requirements.

Requirements in Pregnancy and Lactation. All the evidence points to an increased requirement of vitamin C in pregnancy and lactation. The early studies of Neuweiler [417] showed that it was necessary for pregnant women and nursing mothers to ingest considerably larger amounts of test doses of vitamin C than normal adults to maintain excretion levels indicating an adequate intake. More than half of a group of pregnant women examined by Gaetgens and Werner [418] were receiving insufficient vitamin C, as tested by the saturation technique of Jezler and Kapp [861] (p. 546). Vitamin C plasma values tend to be lower than normal and fall towards the end of pregnancy [391, 397, 421, 422, 989]. Teel and his colleagues [391] also found that the vitamin C of the blood is not as easily maintained at high levels by supplements of vitamin C as it is in non-pregnant women.

Using the saturation test previously described (p. 515) Widenbauer [876] estimated the requirements of a pregnant woman at 70 mg. a day in the third and 67 mg. a day in the eighth month of pregnancy; using the same technique a non-pregnant control only required 28 mg. These figures agree with those of Olinger and Menczel [419], who found that an average of 68 mg. of vitamin C a day was necessary to maintain a state of saturation in a group of a hundred and seven pregnant women. Using saturation tests Gaetgens [420] obtained values ranging from 33 to 64 mg. a day for the pregnant woman, and he recommends not less than 100 mg. a day as a safe requirement. From a study of the vitamin C excretion in response to test doses in pregnant and nursing women, Toverud [428] concluded that the daily intake of vitamin C should not fall below 75 mg. a day in the case of pregnant women. Javert and Stander [989] estimate the requirements as 200 mg. daily. The National Research Council (U.S.A.) allowance is 100 mg. in the latter half of pregnancy.

Lund and Kimble [979] have carried out a detailed investigation on vitamin C nutrition in pregnancy. They find that if the mother receives an adequate diet, the mean value of the plasma vitamin C is 0.95 mg. per

cent., on a "fair" diet 0.52 mg., and on a poor diet 0.18 mg. Marked seasonal changes were noted, the values being higher in summer. Lund and Kimble found that adequate blood levels could be maintained on a good diet without recourse to supplements of synthetic vitamin C. A good diet included freshly-cooked greens which are excellent sources of vitamin C (p. 448). The blood levels were slightly lower in the early puerperium, due probably to secretion in the milk. Lund and Kimble point out that hyperemesis may lead to a dangerously low intake of vitamin C and to clinical scurvy. They believe that the retinal hæmorrhages of severe hyperemesis gravidarum are a manifestation of vitamin C deficiency and state that they cease after vitamin C therapy. On rare occasions a deficiency of vitamin C may lead to post-partum hæmorrhage, although generally there is no connection between them.

The nursing mother needs enough vitamin C to satisfy not only her own requirements, but also those of her child. Various studies on the vitamin C content of human milk show that there is an upper limit or threshold value, about 8 mg. per 100 c.c., which cannot be exceeded, however much vitamin C is given to the mother. Baumann and Rappolt [898, 424] have calculated that a nursing mother secreting 800 c.c. of milk a day, containing at least 5 mg. of vitamin C per 100 c.c., requires a minimum of 50 mg. of vitamin C beyond the normal daily requirements, *i.e.*, 100 to 125 mg. a day. After birth the breast-fed infant may obtain adequate vitamin C, even if the mother is on a diet deficient in the vitamin, by the depletion of her body reserves. Toverud [428] found that 75 to 100 mg. a day was necessary to maintain a state of saturation in ten nursing mothers; this raised the vitamin C content of the milk from 6 to 7 mg. per 100 c.c. He concludes that in order to provide the breast-fed infant with 25 to 40 mg. of vitamin C a day the mother should receive an intake of 100 mg. a day. Elmby and Becker-Christensen [421] consider that an additional 100 mg. of vitamin C a day is needed during lactation, since the administration of this amount brings the plasma vitamin C up to normal levels. Chu [896], who has studied the vitamin C requirements of the nursing mother in China, concludes that the daily requirements are approximately 82 mg. of vitamin C for a mother of 45 kilos. The Western woman, who weighs on an average about 60 kilos in the early puerperium, would be expected to require quite 110 mg. of vitamin C a day on this basis. Making an allowance of 40 to 50 mg. a day for the infant Widenbauer [410] considers that the nursing mother requires 80 to 100 mg. daily.

It is probably safe to assume from the above data that the nursing mother requires from 100 to 150 mg. of vitamin C daily. The allowance of the National Research Council (U.S.A.) is 150 mg. daily.

According to Neuweiler [860] there has been a considerable drop in the level of vitamin C nutrition during the war in pregnant and lactating women on the Continent. Before the war blood levels among Swiss women were of the order of 0.65 to 0.91 mg. per 100 c.c.; in 1942 the level dropped to 0.36 to 0.53 mg. Investigations in Britain in 1944 show that the level of vitamin C nutrition in pregnant women is not as good as it might be [998].

Requirements and Exercise. It was frequently noted by the early

explorers and seafarers that the most active members of the party or crew suffered from scurvy first. It has been implied from this that the requirements of vitamin C are increased by exercise. Experimental evidence concerning the effect of exercise upon the metabolism of vitamin C is, however, inconclusive. Van Eekelen found a decreased level of vitamin C in the liver and suprarenals of rats that had been made to do forced work to the point of exhaustion, and Hamel [888] records a lowered excretion of vitamin C after violent exercise and sports. Hamel was led to believe that an intake of 85 mg. of vitamin C a day was insufficient for an adult engaged in moderate athletic action, which increased the needs to 45 to 55 mg. a day. According to Hamel an inadequate intake of vitamin C results in a marked decrease in efficiency. Jezler and Haffter [885] noted a sharp decline in the urinary excretion, which persisted for two days, after prolonged exercise, such as an exhausting run on skis. Wiebel [886] tried the effect of giving additional vitamin C to girl athletes in one group and blank tablets in a control group. The findings are of doubtful value because the level of vitamin C nutrition before the experiment was not known. Conflicting results were also obtained by Brandt [887], who noted that muscular effort sometimes increased and sometimes decreased the urinary output of vitamin C. Brunner [846] states that during a period of heavy marching with full kit Swiss soldiers needed 200 mg. of vitamin C daily. Such large quantities were stated to make the men feel fresher, stronger, better able to stand exertion and prevent muscular pains in the legs. Du Pain and Loutifi [990] report freedom from fatigue in subjects doing severe physical exertion receiving doses of 750 to 2,000 mg. in a few hours. Much of this work on vitamin C and exercise was uncontrolled. Not only should controls be used in such tests, but the subjects themselves should serve as their own controls by being given blank tablets or injections of saline, otherwise psychological effects which are so important in exercise and fatigue tests, cannot be ruled out.

It is difficult to reconcile the foregoing observations with those of Rietschel and Mensching [888], who existed for a hundred days on a diet deficient in vitamin C, yet they felt fit and active, although the blood values for vitamin C were practically zero. Fox [844] and his co-workers have drawn attention to the fact that South African mine labourers exist on a vitamin C intake of 15 mg. a day—far below the minimum figure accepted by most authorities—yet they engage in hard work without showing fatigue. Four independent lines of investigation failed to demonstrate that further additions of vitamin C to the diet of the natives lead to any noticeable improvement in muscular efficiency. Recent carefully controlled work by Johnson and others [1091] has shown that two months' deprivation of vitamin C leads to no detectable deterioration in physical vigour, nor do supplements of 75 mg. of vitamin C daily produce any detectable benefit in manual workers with respect to well-being, physical vigour and efficiency.

Faulty Absorption. It has been shown that in certain gastro-intestinal conditions appreciable amounts of vitamin C fail to be absorbed from the gastro-intestinal canal (p. 498). An increased intake is necessary to compensate for this. Bercovitz and Page [991] have shown that

vitamin C deficiency occurs in ulcerative colitis in spite of an intake sufficient to maintain saturation in a normal person.

Effect of Drugs. The administration of certain drugs, such as aspirin, atropine and ammonium chloride and anaesthetics, increases the excretion of vitamin C (p. 508), and may therefore increase the requirements.

Requirements and Infections. Clinically a diminished urinary excretion of vitamin C has been observed in a number of infections, including pneumonia [482], diphtheria [483], osteomyelitis [484], rheumatic fever [485], rheumatoid arthritis [486], tuberculosis [487]. Faulkner and Taylor [488] showed that whereas the serum vitamin C of healthy persons averages 1.81 mg. per 100 c.c., patients suffering from acute infections present values of the order 0.65 mg. Several investigators have attempted to show that this increased utilization is due to the raised metabolism resulting from the pyrexia since they state that artificial pyrexia produces a similar effect [425-430]. Recent investigations by Osborne and Farmer [992], who submitted subjects to temperatures of 104° F. in fever cabinets, fail to confirm this. They observed no significant change in the vitamin C blood level before or during pyrexia. Abt and his co-workers [993] also concluded from studies on scarlet fever, rheumatic infections and diphtheria that pyrexia alone does not affect the vitamin C reserves or utilization.

The work of Tonutti and his co-workers [102, 108] suggests that additional vitamin C is required as a direct result of infection, because if animals are experimentally infected with pneumonia, tuberculosis or diphtheria a few hours after infection there is a rapid migration of vitamin C from the tissues into the leucocytes, which rapidly become saturated with the vitamin. In pneumonia the alveolar exudate is full of phagocytes laden with granules of vitamin C (p. 481). According to Tonutti the increased utilization of vitamin C observed in so many febrile states occurs at the infective focus, *e.g.*, in the alveoli of the lungs in pneumonia. The relationship between vitamin C and infection is fully discussed on p. 476.

Requirements and Diseases with Raised Metabolism. There appears to be increased utilization of vitamin C in diseases associated with raised metabolism resulting from an increase in oxidative processes or in cellular proliferation. A decreased excretion of the vitamin indicating increased utilization is found in patients suffering from hyperthyroidism [439], malignant disease [440] and leukaemia [474]. Minor and Ramirez [440] explain the increased utilization in cancer patients on the assumption that cellular respiration is greater in cancerous tissue.

Gosh [994] has shown that drugs increasing general metabolism, such as thyroid, 2 : 4-dinitrophenol and insulin increase the excretion of vitamin C and deplete the tissue storage of the vitamin in experimental animals.

Requirements in Relation to Race and Climate. While it is not considered that the human requirements are subject to seasonal variations there is evidence that the intake of vitamin C is subject to seasonal fluctuations. Thus in Sweden Dagulf [879] found an average serum vitamin C of only 0.22 mg. per 100 c.c. in the spring and 0.9 in the summer for three hundred and twenty-six healthy persons. Neuweiler [884] also states that in Bern the average intake of vitamin C is 30 to 35 mg. in the winter and 50 to

55 mg. in the summer. Harris [893] records similar variations in England. The lower intake in winter and spring is due to the fact that foods rich in vitamin C are relatively scarce in the winter months. In the summer a greater range of fruit and vegetables is available. Göthlin has suggested that this limitation of foodstuffs containing vitamin C by climatic factors may actually affect the human requirements of vitamin C. This may result in reduced urinary excretion of the vitamin and more efficient utilization. Some degree of body synthesis cannot be excluded (p. 512). Levels of vitamin C nutrition which have been shown to result in scurvy in England are tolerated by natives of South Africa who do not get scurvy on intakes of 15 mg. a day or even less [844, 847]. It is also well known that certain native tribes, such as the Xosa people of South Africa, regard the consumption of green vegetables as beneath the dignity of the male sex and therefore refuse to consume one of the most important sources of vitamin C. Yet the males do not appear to be any more scorbutic than the women of the tribe [847]. Fox [844] has tried the effect of increasing the vitamin C intake of native South African mine labourers receiving 12 mg. a day in their food, with supplements of 40 mg. a day in the form of orange juice, but he could detect no significant improvement in general health, physical efficiency or resistance to infection.

According to Henschel and his co-workers [1071] the requirements of vitamin C are not raised by a high environmental temperature, or at any rate the administration of additional vitamin C appears to have no significant beneficial effect on the ability of subjects to perform muscular work at high temperatures. The performance of forty-four young men doing muscular work at a temperature of 122° F. for several hours was studied first on a diet poor in vitamin C, and then on one supplemented by 500 mg. of vitamin C daily. Pulse rate at rest and at work, rectal temperature, vasomotor stability tests, rate of sweating, subjective feelings, psychomotor tests and strength tests were independent of the intake of vitamin C, as also were the daily loss of sweat and the incidence of heat exhaustion. There therefore appears to be no advantage in giving supplements of vitamin C under hot climatic conditions. Similar results were obtained using a mixture of vitamins B₁ and C, nicotinamide and riboflavin [1076].

Composition of Diet. Patterson and Bourquin [980] suggest that a high protein diet increases the requirements of vitamin C, as judged by saturation tests. Chakraborty and Roy [504] found that a high protein diet results in a decreased excretion of vitamin C. The protein may not affect the utilization of vitamin C at all, but alter the renal threshold for the vitamin.

DISEASES ASSOCIATED WITH VITAMIN C DEFICIENCY SCURVY

Ætiology. Scurvy is a deficiency disease considered to be due, partly at any rate, to an insufficient intake or absorption of vitamin C. It has been maintained that lack of this vitamin is not the sole causative factor, since cases of scurvy have been described resistant to treatment with pure

vitamin C [441-448]. Some of these cases, however, responded to parenteral but not to oral treatment with the vitamin. Mawson [444] has described a case of scurvy in which the vitamin C intake was adequate, although the excretion and the blood level were low. "Renal scurvy" was diagnosed, implying that the patient had an abnormally low renal threshold for vitamin C and was therefore unable to store sufficient in the tissues to prevent scurvy.

With the discovery of other vitamins in fruit juices, particularly vitamin P, it has been suggested that scurvy, like beriberi and pellagra, is a multiple avitaminosis. According to Szent-Györgyi and Scarborough (pp. 857, 862) scurvy is a disease due to a combined deficiency of vitamin C and P. Todhunter [87] has reported that the antiscorbutic value of lemon juice is greater than that of the vitamin C that it contains. He considers that lemon juice may contain another factor besides vitamin C, which is concerned in the prevention of the hæmorrhage of scurvy. Cameron and Mills [1015] gave vitamin P but not vitamin C to a case of classic scurvy. The hæmorrhagic features promptly disappeared, but the other manifestations were unaltered.

How long does it take for a healthy adult to become scorbutic when fed on a diet otherwise adequate but devoid of vitamin C? There have been various answers to this question. According to Lind it occurs between four and six months at sea on a scorbutic diet. Mensching [342, 388] lived on a diet poor in vitamin C for a hundred days without getting scurvy. Crandon [68], who lived on a diet adequate in all respects, but lacking vitamin C, observed that scurvy developed after a hundred and thirty-two days of total vitamin C deficiency. Symptoms of scurvy appeared in a group of Chinese refugees after three to eight months on a deficiency camp diet of cereals and salt turnip [884]. In infants scurvy develops between the second and ninth month [514]. Scorbutic symptoms occur in monkeys after seven months on a scorbutic diet [505]. Farmer [1078] observed no clinical evidence of scurvy, except hyperkeratotic papules surrounding the hair follicles on the legs, in volunteers on a scorbutic diet (0 to 10 mg. vitamin C daily) for five months.

Factors responsible for the actual appearance of scurvy in addition to a scorbutic diet are climate, composition of the food in relation to other vitamins and minerals, constitution of the subject, condition of internal secretions, presence of infection, and the condition of the alimentary tract. According to Lund [460] patients with gastric lesions have low reserves of vitamin C and are often near the scorbutic level. The causes of scurvy met with in the population at large are "ignorance, apathy and poverty" [1041]. According to McMillan and Inglis [1041] there has been an increase in scurvy since the beginning of the war. In 1941-42 scurvy formed over three per cent. of all admissions to medical wards in an Edinburgh municipal general hospital. According to the Ministry of Food the vitamin C intake of Edinburgh and Glasgow is much below the average for all towns in Britain. McMillan and Inglis attribute the relatively high incidence of the disease firstly to ignorance, particularly in males living on their own ("bachelor scurvy"), of the need for potatoes and vegetables in the diet. Secondly to apathy because such foods require

preparation and cooking, and thirdly to poverty, making it impossible to buy an adequate diet.

Clinical Manifestations of Scurvy. The adult suffering from scurvy complains of weakness and pain in the limbs. Exaggeration of patellar and biceps reflexes has been described [884]. Multiple minute hæmorrhages occur all over the body, particularly about the hair follicles of the lower extremities, which are brawny and tender.

As navigators and explorers have observed, weakness is one of the early complaints of scurvy. This is associated with fatigue on exertion, palpitation and breathlessness. The patient sits, rather than stands or walks, and when he does stand he flexes his legs. Hæmorrhage from the gums and stomato-gingivitis, hypertrophy of the dental papillæ (see Fig. 143), followed by loosening of the teeth are common symptoms of scurvy; the loosening of the teeth is due to the resorption of the alveoli of the jaw bones. One of the most reliable early signs of scurvy is gingivitis. The characteristic swollen boggy gums of scurvy occur only when teeth are present and are most marked about defective teeth (Figs. 143, 144). Not only may hyperæmia and hæmorrhage of the gums occur, but epithelial degeneration, ulceration and even gangrene. Owing to the infection around the base of the teeth the breath is fœtid. Other hæmorrhagic manifestations include hæmaturia, melæna with diarrhœa, pin-point hæmorrhages in the gut with subsequent ulceration, menorrhagia, metrorrhagia, epistaxis and subperiosteal hæmatomata. The latter cause the limbs to be painful to the touch. Extravasations of blood from larger vessels into the muscles may occur (Fig. 146), as well as beneath mucous membranes, in the gums, conjunctivæ and lining of the serous cavities and joints. Petechial hæmorrhages are also present (Fig. 145). Farmer [1078] observed petechiæ following slight trauma in volunteers kept on a scorbutic diet for five months.

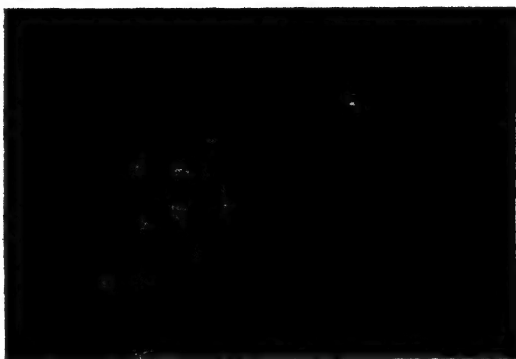


FIG. 143. Scorbutic Gingivitis. The patient is a child on a very poor diet in a Scottish Episcopalian orphanage. Hæmorrhage from the gums and hypertrophy of the dental papillæ are clearly seen. The teeth appear to be healthy, although irregular.

The hæmorrhagic manifestations have been stated to be due to abnormal capillary fragility, although morphological changes have not been detected in the capillaries and small blood vessels, and more modern work negatives any connection between vitamin C deficiency and increased capillary fragility (p. 589). Scarborough [86] believes that vitamin P may play a part in the hæmorrhagic manifestations of scurvy. Only a third of all cases of scurvy show an increased capillary fragility [1088].

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Anæmia, which is usually orthochromic or hypochromic and normocytic, is a characteristic finding, although it is not always present. It is responsible for the pallor, shortness of breath and palpitations seen in the scorbutic patient. There is often diminished erythropoiesis in the bone marrow, which is restored to normal by giving vitamin C [999]. The anæmia of scurvy is probably not due to vitamin C deficiency alone, as lack of the vitamin does not affect blood formation [68]. It is also frequently relieved by iron therapy alone [110].

Infection with its resulting pyrexia is common. The depleted vitamin C reserves of the patient suffering from scurvy are said to render him more susceptible to infection. If death occurs it is due to intercurrent infection, *e.g.*, bronchopneumonia, or it suddenly occurs with syncope. Crandon [68], however, contests these views. Although he suffered from frank clinical scurvy for a month and subclinical scurvy for several months, he remained free from infection, with the exception of "colds." His work, published in 1940, is such a valuable contribution to the study of human scurvy and vitamin C deficiency that a detailed account of it is given here.

Crandon placed himself on a diet free from vitamin C, but containing all the other essentials, including a complete range of all the other vitamins and minerals. Samples of blood were repeatedly tested in various ways and he was kept under clinical observation. During the first four months all the clinical findings were negative. There was a slight loss of weight, a fall in the metabolic rate and a feeling of weakness and fatigue on slight exertion. It was only after a hundred and thirty-four days had elapsed that clinical manifestations of scurvy appeared. The loss in weight, which eventually reached a maximum of 27 lb., was attributed to the monotonous diet rather than to the absence of vitamin C.

Skin Lesions. After a hundred and thirty-four days had elapsed small hyperkeratotic papules began to develop over the buttocks and posterior aspects of the calves; there was also noticeable fragmentation of hairs. These lesions resembled the follicular keratitis of vitamin A deficiency. Each papule contained an ingrown hair, which could be seen if the hyperkeratotic plug was picked or scraped off, leaving a small slightly bleeding crater. There was also marked dryness of the skin, particularly over the extensor surfaces and backs of the hands. The existence of vitamin A deficiency was ruled out because 80,000 units of vitamin A were being taken daily and biophotometer readings and plasma vitamin A values were within normal limits.

After a hundred and sixty-one days small perifollicular hæmorrhages or petechiæ appeared on the legs, particularly after standing for long periods. After six months they were abundant over the whole of the leg, and on the thighs took the place of the hyperkeratotic papules. The administration of vitamin C rapidly cleared these lesions.

Wound Healing. The experiments of Crandon and others on the healing of wounds have been previously described (p. 468).

Teeth and Gums. During the first five months no changes were observed in the teeth or gums. At the end of six months the gums were slightly boggy on pressure, but no other gross changes were observed. A biopsy of the gums showed normal tissue. It is interesting to note, how-

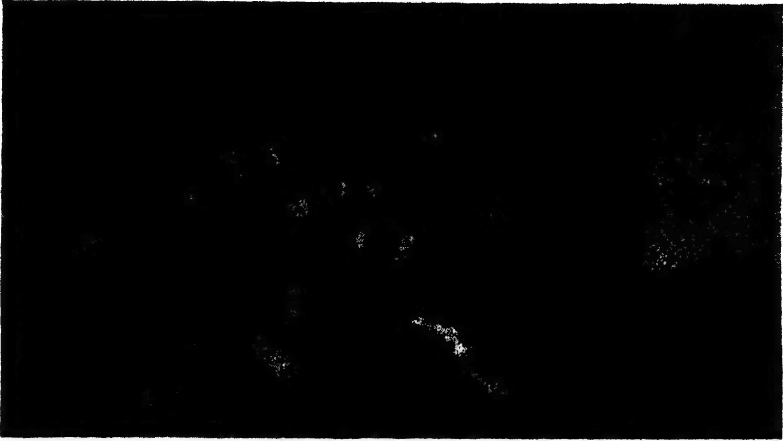


FIG. 144. Scurvy. A patient with scurvy showing considerable swelling, sponginess and discoloration of the gums.

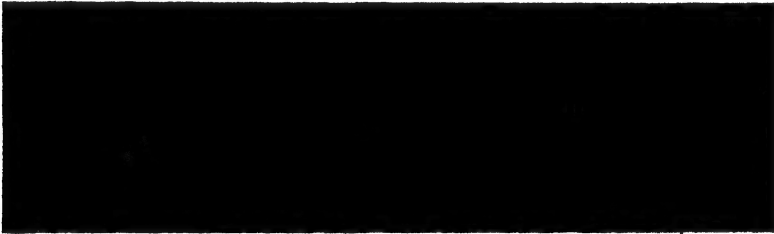


FIG. 145. Scurvy. Forearm of the same patient as in Fig. 144 showing bleb-like extravasations of blood resulting from slight traumata a year previously. Petechial hæmorrhages resulting from a recent tourniquet test are also seen.

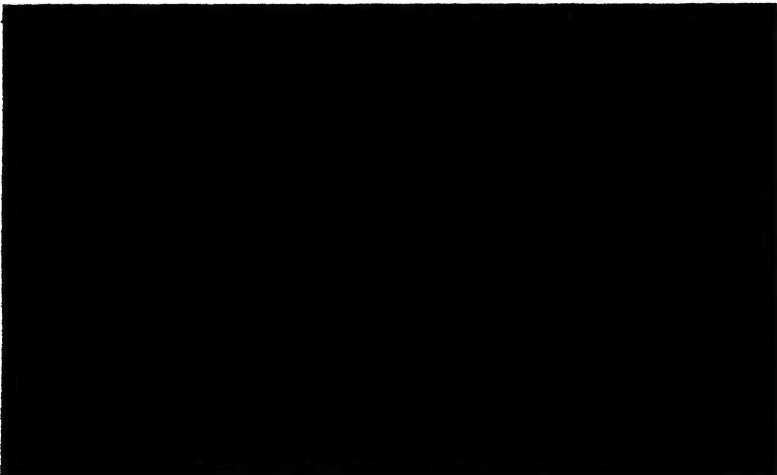


FIG. 146. Scurvy. The same patient as in Fig. 144, showing extravasation of blood around right ankle and on right shin. Some oedema of the foot is present.

ever, that X-ray films of the teeth showed occasional interruptions of the lamina dura. No trace of bleeding occurred on brushing the teeth.

Farmer [1078] kept several volunteers on a scorbutic diet (0 to 10 mg. vitamin C daily) for five months, but failed to observe any changes in the gums, teeth or jaws, either with the naked eye, radiologically or with the biomicroscope.

Hæmatological Picture. Although blood was lost by venesection on four occasions and as a result of the numerous blood estimations no anæmia developed. The percentage hæmoglobin showed a slight fall during the third month of the diet, but was due to iron deficiency since it was corrected by the administration of ferrous sulphate. These observations are in accord with those of other workers in this field (p. 475).

The white cell count averaged 5,000 at the beginning of the experiment and no appreciable change was observed until after a hundred and thirty-two days, when the count fell to 3,500 and later to 3,200. Following the administration of vitamin C, the count shot up rapidly to 5,000 and then to 9,000.

In experimental scurvy the prothrombin time is not appreciably increased [1049].

Capillary Fragility. The capillary fragility test as determined by the technique of Göthlin (p. 539) remained negative. At the end of five months, when frank scurvy was present, there were fewer petechiæ on the arm after applying a blood pressure cuff for ten minutes at 100 mm. mercury than there were in normal controls. The negative pressure method of Dalldorf (p. 540) also gave negative results. This is in keeping with the most recent work on the subject, which negatives any connection between vitamin C deficiency and increased capillary fragility (p. 541).

Blood Pressure. This remained constant at 120 systolic, 70 diastolic, except on one occasion when it dropped temporarily owing to a large loss of blood.

Resistance to Infection. Contrary to expectations based on the work on vitamin C and infection (pp. 476-481) there was almost complete freedom from respiratory infection, even though the experimental period included the winter months. Blood complement determinations were normal throughout the period of observation even when frank scurvy was apparent.

Fatigue. Fatigue appeared at the beginning of the third month and became progressively more marked. Careful tests after prolonged vitamin C deficiency showed impaired capacity for aerobic work, e.g., walking and running on the treadmill. No great change could be detected in the capacity for doing anaerobic work, e.g., muscular movements measured by an ergograph. Tests of harder grade work—a run to exhaustion at seven miles an hour—showed that running was only possible for sixteen seconds, whilst following the administration of vitamin C the time was increased to sixty-six seconds and to eighty-four seconds after normal diet had been resumed. The performance whilst in the scorbutic state was equivalent to that of a man in the eighth decade of life. Another interesting observation was that after a period of aerobic work in the scorbutic state the rate of disappearance of the blood lactate was extremely slow.

Crandon has drawn attention to the fact that scurvy might have appeared much sooner if he had been submitted to extreme muscular fatigue. After it had been decided to terminate the experiment, fatigue and languor were considerably diminished within twenty-four hours of administering vitamin C.

Farmer [1078] has also studied fatigue phenomena in young volunteers kept for five months on a scorbutic diet (0 to 10 mg. vitamin C daily). A measurable decrease in work output occurred and the subjects complained of severe fatigue after three months' on the diet. Errors in "choice reactions" were increased and there was loss of interest in work and motivation. Threshold of perception, co-ordination of motion on a pursuit meter and critical fusion frequency of visual flicker showed little or no change from the normal.

Blood Vitamin C. The plasma vitamin C rapidly fell and reached zero after forty-one days on the diet, remaining at this level for thirteen weeks before the first evidence of clinical scurvy appeared. On the other hand, the vitamin C level in the white cell platelet layer of the centrifuged blood fell gradually from a relatively normal level of 28 mg. per 100 c.c. on the seventieth day to 4 mg. on the eighty-second day, but did not reach zero until shortly before the appearance of clinical scurvy. These observations and the fact that normal wound healing occurred after three months of vitamin C deficiency, when the plasma vitamin C had been zero for forty-four days, suggest that plasma vitamin C values are a poor index of the vitamin C status of the individual. As pointed out by Butler and Cushman (p. 552) the white cell platelet level of vitamin C in the centrifuged blood is a more accurate measure of the degree of vitamin C deficiency than plasma determinations.

Miscellaneous Observations. The basal metabolic rate fell at one time as low as minus twenty-two per cent. This was probably due to loss of weight, or inanition, or both, rather than to vitamin C deficiency. Insulin and glucose tolerance tests were normal. Gastric analysis revealed a large drop in the free and total acid; this was restored after administration of vitamin C. There was a lowering of the total phosphorus content of muscle, and an increase of the phosphagen phosphorus. All other tests on blood, urine, the stools, and X-ray films and electrocardiograms were within normal limits.

The opinion is expressed that the long interval that elapsed before clinical scurvy appeared was probably due to the absence of complicating factors, such as growth, infection or multiple avitaminosis. The study proves that clinical scurvy as ordinarily met with is undoubtedly a multiple deficiency disease, whereas Crandon was suffering from severe uncomplicated vitamin C deficiency. It has been observed elsewhere (pp. 192, 394) that simple vitamin deficiencies do not exist except experimentally.

Fox [844] and his colleagues studied nearly a thousand mine labourers in Africa on diets low in vitamin C—generally about 15 mg. a day. Some of their findings can be correlated with those of Crandon. For example, no changes in capillary fragility were detected; there were no signs of anæmia; the gums remained healthy; resistance to infection was not diminished; the healing of wounds was not impaired unless vitamin C

was *totally* withheld for six months. Even when the blood vitamin C was nil there was no sign of scurvy. Fox and his co-workers concluded that deprivation of vitamin C unless extremely severe and prolonged, does not of itself lead to the appearance of scurvy in the absence of precipitating factors, such as infection.

Infantile Scurvy. This is still seen in children, in spite of general improvements in infant feeding. Under the names of *Barlow's disease* and *scurvy rickets* it was frequently observed a few years ago. Scurvy rickets is a misnomer as a separate entity; scurvy and rickets may occur in the same child. For the reasons previously given (p. 514) infantile scurvy is more prevalent in the artificially fed infant than in the breast-fed, particularly if the infant is given overboiled, dried or condensed milk. The disease usually makes its appearances in children of two to twelve months, and is rare after the second year. Faulkner [835] states that the incidence of scurvy among children admitted to the Boston City Hospital is 0.14 per cent.

The onset of the disease is gradual and the child is usually brought to the family doctor because he screams when his limbs are touched, because he does not use his limbs, or because of bruising of the orbit and limbs, and hæmorrhage from the gums, bowel or urinary tract. On examination the position of the legs is characteristic; they are flexed, widely abducted and externally rotated (Fig. 148). This position is largely due to the extreme pain caused by subperiosteal hæmorrhages, which are most likely to occur around the bones of the legs. A history of improper feeding is difficult to obtain, as every mother, however ignorant, considers her child well fed. Careful interrogation of the mother on the question of diet is therefore necessary.

On examination the infant is usually wasted, pale and fretful and is terrified of being touched. There may be bruising on the face or body (Fig. 148) and on deep palpation acutely tender swellings may be felt deep to the muscles, particularly of the leg. There is a failure to gain weight—which may be masked by œdema—weakness, rapid shallow breathing, a rapid pulse, diarrhoea and vomiting. If there is retrobulbar hæmorrhage the eye may be proptosed. The temperature is usually slightly raised, about 100° F.; the fever in both clinical and experimental scurvy has never been satisfactorily explained, but is probably due to the absorption of blood from the hæmorrhages. Hæmorrhage from the gums (Fig. 147), bowel or urinary tract may be observed, but the mouth lesions do not occur if the infant is edentulous (*cf.* adult scurvy, p. 525). The gums are swollen, red and dusky if the teeth are due to erupt. Intracranial hæmorrhage has been reported.

Moderate secondary anæmia is often present with hæmoglobin levels in severe cases as low as forty per cent. of normal. Examination of the blood and urine shows the absence of vitamin C or markedly reduced levels. A few red blood cells in the urine are of diagnostic importance.

There are no essential differences between adult and infantile scurvy. In both varied manifestations of a hæmorrhagic tendency are the prominent features.

In its early stages infantile scurvy can be diagnosed radiologically

INFANTILE SCURVY

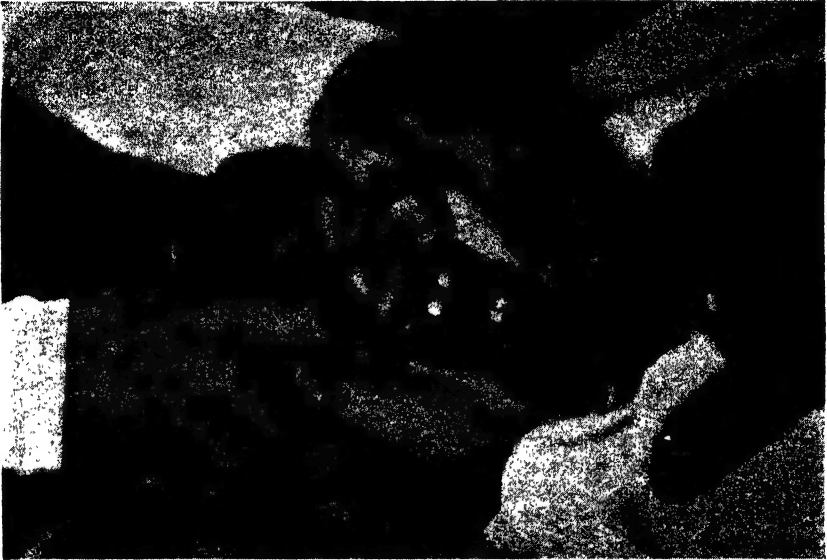


FIG. 147. Infantile Scurvy. Swollen, spongy and hæmorrhagic gums in a child of 10 months suffering from scurvy.



FIG. 148. Infantile Scurvy. The same child as in Fig. 147. There is gross hæmorrhage under the periosteum of the femora ; both legs, which are in the characteristic position of flexion, abduction and external rotation, are swollen and tender ; and there are hæmorrhages into the tissues of the legs and scrotum.

INFANTILE SCURVY

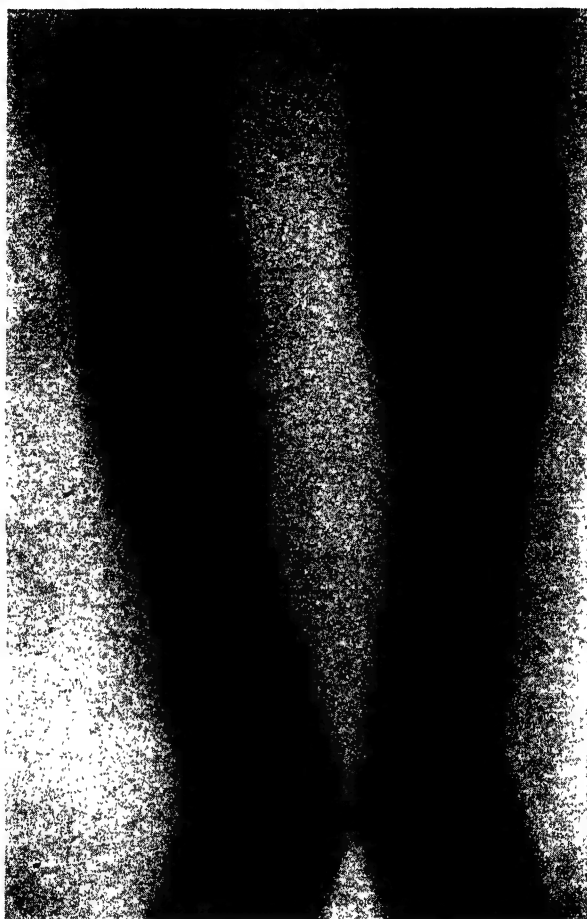


FIG. 149. Infantile Scurvy. Radiogram. Note decalcification of the epiphyses with a well-defined periphery ; calcified subperiosteal hemorrhages, which show as a dark shadow on the medial side of the tibia and between the tibia and fibula of the left leg ; and the dense metaphyseal borders of the diaphyses.

(Fig. 149). As a result of the observations of Pelkan, Fraenkel, Baetjer, Wimberger, Schwartz and Bromer [500] ten X-ray signs have been recognized :—

(1) A zone of rarefaction immediately posterior to the zone of preparatory calcification ; the “scurvy line,” the framework marrow (Bromer), the *Gerüstmark* of the German writers.

(2) A broad, finely irregular edge of dense shadow around the centre of ossification, with rarefaction of the central portion (Wimberger's sign).

(3) A finely irregular broadened intensely calcified zone of preparatory calcification at the epiphyseal end of the long bones, the so-called “white line of Fraenkel.”

(4) A small spur at the lateral edge of the epiphysis (Pelkan).

(5) Separation of the epiphyses.

(6) A ground glass transparency of the shaft, with clouding or obliteration of the trabecular structure visible in normal bones.

(7) Thinning of the cortical shadow, often represented by a narrow white line.

(8) Subperiosteal hæmorrhages, occurring only in the late stage (Fig. 149).

(9) Subperiosteal fractures in the ends of the diaphysis.

(10) Enlargement of angulation of the costochondral and vertebral junctions of the ribs.

Taken alone the majority of these signs are not pathognomonic. Signs (2), (3) and (4) are not absolutely characteristic of scurvy and may also be seen in rickets, lead and phosphorus poisoning and after administering vitamin D preparations. The most important sign is (1).

There are three signs said to be diagnostic in latent scurvy, known as Pelkan's triad. They are : (a) a broadened epiphyseal line ; (b) a dense shadow around the centre of ossification of the epiphysis ; (c) absence of trabeculations in the shaft. Park [514], on the other hand, states that there are no early radiological signs, and that reliance must be placed on early clinical signs.

The healing of scorbutic bone can be demonstrated radiologically after two weeks' treatment (Pelkan [500]). At first the broadened epiphyseal line loses its finely irregular appearance and becomes sharply outlined. The scurvy line not only disappears but becomes more heavily calcified than the rest of the shaft. Years after clinical cure radiological examination still shows oval, definitely circumscribed areas of rarefaction in the interior of the epiphyseal centres of ossification [1000].

Morbid Anatomy and Pathology of Scurvy. The principal lesions are hæmorrhagic and skeletal. The former have already been described. The skeletal lesions resemble those of the guinea-pig, the commonest sites being at the costochondral junctions, the distal ends of the femora, and the proximal ends of the tibiæ and femora and the wrists. The lesion is characterized by rarefaction of the cortex and conical widening of the bone, inhibition of bone growth and replacement of the normal junction of bone and cartilage by a zone of connective tissue poor in collagen and containing fragments of densely calcified cartilage with no osteoid tissue. These

changes result from the inability of the osteoblasts to form normal osteoid tissue in the absence of adequate vitamin C, with consequent attempts at fibrous tissue union between the epiphysis and diaphysis. Epiphyses may be separated and fractures occur.

The dental lesions in human scurvy include hyperæmia and œdema of the pulp, degeneration of the odontoblast layer with cyst formation, destruction and calcification of vessels and necrosis and calcification of areas in the pulp. The dentin becomes porotic and resorption occurs along Tomes' canals, which widen into spindle shaped and round spaces. Abnormal and irregularly canalized dentin is formed. The commonest lesions are in the apical third of the tooth. Lesions may occur in the teeth before they do in bone. The loosening and falling out of the teeth in advanced scurvy is due to the rarefaction of the alveolar bone.

The gingival lesions consist of hyperplasia of the papillæ, development of granulation tissue and finally gangrene; they do not occur unless teeth are present.

In the older literature on scurvy œdema, particularly around the ankles (see Fig. 146), enlargement of the heart, hydropericardium and hydrothorax were described. Neuritic changes, such as degeneration of the peripheral nerves and large anterior horn cells have been described, as well as sensory disturbances and reduced or absent knee-jerks. Ulceration of the gastrointestinal tract, atrophy of various organs and endocrine glands are also mentioned. It is almost certain that all these changes do not result from vitamin C deficiency; inanition, repeated hæmorrhage, multiple vitamin deficiencies and infection all play a part.

As emphasized by Dalldorf [445] the morphological changes of scurvy are greatly modified by growth, activity, stress, trauma and the presence of complicating factors increasing the vitamin C requirements. Generally speaking the extent and severity of the lesions diminish with age, particularly in the skeleton, the bones most affected being those in which growth happens to be most active at the time of the deficiency. Stress (motion, pressure) exerts an important effect on the site and extent of hæmorrhage. Infections, which increase the vitamin C requirements, may precipitate scurvy in a person bordering on this condition.

In the scorbutic guinea-pig pathological changes occur in the cells of the liver and kidney (Figs. 150 and 151) as evidenced by the deposition of trypan blue in these cells after subcutaneous injection of this dye [1001]. Fatty metamorphosis also occurs in the liver cells. Aschoff and Koch [518] described advanced fatty degeneration in the liver cells in human cases of scurvy, although this may have been due to multiple deficiencies. Beyer [952] observed that fatty degeneration of the liver produced by toxins occurred more readily in scorbutic guinea-pigs than in those receiving adequate vitamin C (Figs. 188-185).

Diagnosis of Scurvy. Clinical diagnosis of mild scurvy is difficult and uncertain. In frank scurvy in the adult the diagnosis is made on the multiple petechiæ, hæmorrhagic manifestations, mouth lesions, extreme fatigue and bad dietary history. In isolated cases the disease may be confused with purpura. Mercurial stomatitis may resemble the oral lesions of scurvy, but it does not present the other features. Acute

PATHOLOGICAL CHANGES IN LIVER AND KIDNEYS IN SCURVY



FIG. 150. Pathological Changes in the Liver in Scurvy. Section of liver of a guinea-pig after intravenous injection of trypan blue dye, showing large fat vacuoles, indicating fatty degeneration, are also present in the liver cells. Control animals showed neither concentration of dye in the liver cells nor fatty degeneration. As trypan blue has an affinity for pathologically altered tissues, these changes are evidence of hepatic damage from vitamin C deficiency.



FIG. 151. Pathological Changes in Kidney in Scurvy. *A* is section of renal cortex of a scorbutic guinea-pig after intravenous injection of trypan blue, showing large granular masses of dye in the cells of the proximal convoluted tubules. *B* is a section of the renal cortex of a control animal showing only slight amounts of dye. As trypan blue has an affinity for pathologically altered tissues, the changes are evidence of renal damage in vitamin C deficiency.

leukæmia should be considered in the differential diagnosis; here the blood count is diagnostic.

Diagnosis in the infant is not always easy, although the typical drawn up and immobile appearance of the legs (p. 148) and the acute pain on touching the limbs are characteristic. The finding of a few blood cells in the urine is a most valuable sign. Osteomyelitis, with its painful limbs, and poliomyelitis, with its immobility, may cause some confusion, although high fever is against the diagnosis of infantile scurvy, and in the latter there is no true paralysis or neurological disturbance. Rheumatic fever, which is, however, rare below the age of two, syphilitic epiphysitis, and Parrot's disease (syphilitic pseudoparesis) may have to be considered. An X-ray of the bones and vitamin C deficiency tests will help to establish the diagnosis.

Special Tests for Scurvy. A number of tests for the detection of vitamin C deficiency have been devised. These are described later (p. 589).

Treatment of Scurvy. Treatment in uncomplicated cases consists of the administration of fruit juices and large doses of vitamin C, *e.g.*, 200 to 300 mg. two or three times a day by mouth, taken preferably before or with meals, since gastric acid probably assists in absorption. There is no need to give vitamin C parenterally unless there is nausea or vomiting or the patient has gastro-intestinal lesions likely to interfere with absorption. The parenteral dosage is somewhat smaller than the oral dose, although 1,000 mg. a day have often been given with good results. It is difficult to overdose with vitamin C. Thus Ralli [847] has given 6 grams intravenously in one dose on several occasions without untoward effects. Intramuscular injections are better than intravenous, since the latter soon spill over into the urine and result in unnecessary wastage. In cases of mild or latent scurvy and infantile scurvy 100 mg. daily by mouth is probably adequate. Fruit juices are also recommended, as cases have been described that are resistant to pure vitamin C. Intensive vitamin C treatment is continued until the lesions are healed and a maintenance dose of 50 to 100 mg. of vitamin C given orally.

Many cases of scurvy are complicated by other vitamin deficiencies, and these should be searched for and treated. An enriched nutritious diet should be given to the scorbutic patient as well as specific vitamin C therapy. The local lesions are treated symptomatically, *e.g.*, mouth washes and mild disinfectants for the mouth to prevent infection; light splints for the legs in infantile scurvy.

Prevention is better than cure in scurvy, particularly in the case of infantile scurvy. This need never occur if infants, particularly the bottle fed, are given fruit and vegetable juices sufficient to supply 20 to 40 mg. of vitamin a day. There is no need to give synthetic vitamin C if natural sources are available.

SUBCLINICAL SCURVY, THE PRESCORBUTIC STATE, SUBVITAMINOSIS C, AVITAMINOSIS C (HYPOVITAMINOSIS C, PARAVITAMINOSIS C, ASYMPTOMATIC SCURVY)

A state of vitamin C deficiency without the clinical manifestations of scurvy has been described under the above names. Diagnosis is not made

on definite clinical grounds, but usually from laboratory tests or from the dietary history. The conception of subclinical forms of scurvy is due to Hess [447], who as far back as 1917 pointed out that an asymptomatic stage of scurvy with well-marked skeletal lesions may precede clinical infantile scurvy. Subclinical scurvy has been reported in guinea-pigs by Mouriquand [448], who showed that the animals can become weak, emaciated and finally die after living on diets low in vitamin C without showing any frank signs of scurvy. It has also been observed that skeletal lesions characteristic of scurvy can be demonstrated in animals suffering from vitamin C deficiency without clinical signs of scurvy [449]. In the human being a group of symptoms characterized by susceptibility to infection, unreasonable loss of vitality, lessened endurance, gingivitis, vague pains, bodily fatigue, loss of appetite and mild digestive disturbances have been attributed, probably incorrectly, to vitamin C deficiency. Certainly a low degree of excretion has been detected in persons showing such a group of symptoms, but, on the other hand, it is known that an individual may excrete very little vitamin C and have low plasma vitamin C values and yet be in excellent health. Dahlberg, Engel and Rydin [1090] carried out carefully controlled observations on five thousand soldiers and concluded that half of them suffered from vitamin C deficiency as judged by urine and saturation tests, yet their health was as good as the other half, who received an extra 50 mg. of vitamin C daily. Zilva [450] strongly deprecates the expressions "hypovitaminosis C" and "subacute or preclinical scurvy" applied to individuals who are unsaturated or excrete low levels of vitamin C. Kassan and Roe [867] also state that they have been unable to recognize subvitaminosis C as a clinical entity, and Hj  rne [1050] has been unable to correlate the general clinical condition of children with their blood vitamin C level.

On the other hand, a large number of children in the poorer classes probably receive suboptimal amounts of vitamin C [887-889] and respond to improved diets [451]. The beneficial effects of the so-called Oslo breakfast, with its fresh vegetables or salad, has been observed by school medical officers. The results may be due to improved nutrition generally and not to an increased intake of vitamin C only. It has been argued that the increased incidence of infection and general fatigue in the spring [451] are due to the gradual depletion of the body's stores of vitamin C over the winter months when fresh foodstuffs are scarce.

Many cases of inflammatory dental disease such as gingivitis have been attributed—probably incorrectly—to lack of vitamin C, and not so much to lack of dental hygiene [453-456]. It is unlikely that the average case of gingivitis is due to vitamin C deficiency (see p. 566).

According to some investigators an  mia is prevalent in states of vitamin C subnutrition. This is difficult to reconcile with Crandon's statement that when he was suffering from frank scurvy he observed no signs of an  mia that did not respond to iron therapy. Lozner [457] has also demonstrated normal regeneration of h  moglobin in patients whose plasma contained no vitamin C.

Deeny [458] believes that vitamin C deficiency plays a part in the causation of the nutritional an  mia present amongst the women of indus-

trial populations and may be a cause of deranged menstrual function, either through its action on the endometrial blood vessels or on the endocrines.

Stapp and Schroeder [517] state that vitamin C deficiency manifests itself as a susceptibility to disease, which leads to definite disease on the appearance of a pathogenic factor which otherwise might have had no harmful effect. Thus infection may precipitate an attack of frank scurvy in a patient whose vitamin C reserves are very low. According to Youmans [751] there is an incidence of some thirty to forty per cent. of latent scurvy in persons whose diets are supposed to be adequate in vitamin C.

Vitamin C deficiency as judged by urine, saturation and blood tests has been detected in a number of patients suffering from various clinical conditions, and there has been a tendency to associate these ætiologically with vitamin C deficiency. This is an uncritical approach because apparent vitamin C deficiency, judged by laboratory tests, can be demonstrated in apparently healthy controls. It is far more likely that in the case of the diseased patient his disease produces a "conditioned" vitamin C deficiency. Thus infections and diseases with increased metabolism may increase the requirements of vitamin C; gastro-intestinal diseases will interfere with its absorption and the patient on a "gastric diet" receives a very low intake; in nervous and mental diseases there is often a restricted intake of food, and hence of vitamin C. The remarks on conditioned vitamin C deficiency in the chapter on vitamin B₁ (p. 206) apply equally well to vitamin C deficiency. Croft and Snorf [521] estimated the blood vitamin C of a hundred unselected patients, and of these thirty-eight were considered to be suffering from vitamin C deficiency as their blood levels were below 0.4 mg. per 100 c.c. Most of them suffered from gastro-intestinal conditions, which undoubtedly conditioned the deficiency. None, however, showed any definite scorbutic signs.

Gingival Manifestations. Kruse [995] states that macro- and microscopic examination of the anterior gums affords a simple, convenient and objective method of detecting vitamin C deficiency. In many cases he says that the gingival manifestations are readily seen naked eye; in some only by biomicroscopy. Both gums are not equally affected, the upper gum often, although not invariably, showing the more advanced process. The sites affected are involved in a definite order: interdental papillæ, the marginal gingivæ, and then the alveolar gingivæ. Kruse divides the changes into acute and chronic, with three stages in each.

In the *acute process* the subsurface vascular papillæ are engorged and dilated, and under the microscope enlarged and congested capillaries are visible. In mild cases this change is restricted to the interdental papillæ and then to the marginal gingivæ. In more severe cases the vascular reaction may be seen all over the gum. At this stage there is no swelling. In the second stage the gum is reddened, first at the points of the interdental papillæ, spreading to the bases and then the marginal gingivæ. In more marked cases the whole gum is intensely red due to the deeper vessels becoming larger and engorged. At this stage there is very little or slight swelling. The subsurface capillary papillæ become less discrete and distinct. In the third stage the reddened gums swell and although the changes may be restricted to the interdental papillæ, more often the

marginal gingivæ are affected and form a red swollen collar projecting around the necks of the teeth. Frequently the entire gum is red and swollen and the swelling may be so intense as to stretch the gum and give it a glossy appearance. The gum may recede slightly from its free edge, increasing the length of the crown, and if it is very swollen a sulcus may form between the gum and teeth, becoming filled with calculus or infected *débris*. Infection of the gum and bleeding gums are common. These changes precede those of frank scurvy in which the gums are congested, bleeding, ulcerated and spongy and are covered with necrotic material. Later the teeth become loose and fall out and the alveolar process becomes necrosed.

In the chronic process the subsurface vascular papillæ show slight dilatation and engorgement, so that gums appear reddened and slightly swollen, the process commencing in the interdental papillæ, extending to the gingival margin and finally over the whole gum. In the second stage the redness and swelling are slowly masked by oedema and filtration, so that the gum appears swollen and pale. The process may be confined to the papillæ or spread over the whole gum. Atrophy, in the form of pitting, occurs in the third stage. The pits or depressions occur first on the interdental papillæ, then on the gingival margins and finally over the whole gum. Although often visible macroscopically the pits are best seen microscopically. In the next stage the atrophy becomes more profound and the pits gradually disappear. The papillæ get smaller and recede from the gum, leaving the cement exposed. Finally the whole gum shows pronounced atrophy, becomes white in colour, and the teeth are loosened and extruded.

Kruse states that these changes are reversible by giving vitamin C. He remarks that in all cases a long period of time is needed for recovery, more than a year in some cases, even with intensive treatment. Using these gingival criteria Kruse states that he has observed a high degree of vitamin C deficiency in America.

Kruse's observations conflict with those of Crandon [68] and Farmer [1078]. After six months on a scorbutic diet Crandon failed to observe any pathological changes in his teeth and gums, although the level of vitamin C in the plasma and white cell layer was zero. This has been confirmed by Farmer and his colleagues [1078], who kept volunteers on a scorbutic diet for five months, but failed to observe any pathological changes in the gums, teeth or bones of the jaw as observed with the naked eye, biomicroscope and X-rays. As in Crandon's case the level of vitamin C in the plasma and white cell layer was zero. Yet Kruse's claims that the gum changes that he has observed are due to vitamin C deficiency, because they can be reversed by giving the vitamin.

LABORATORY METHODS USED FOR DETECTING VITAMIN C DEFICIENCY

Capillary Fragility Tests. Tests involving the measurement of capillary fragility were the first to be used for the detection of vitamin C deficiency. In the *positive pressure method* devised by Göthlin [848], also known as the

Rumpel-Leede test, the exact technique is as follows: A circular area 60 mm. in diameter is marked off in the antecubital fossa and a blood pressure cuff placed at least 2.5 cm. above this and pumped up to 50 mm. mercury pressure, which is maintained for fifteen minutes. After the pressure is reduced the number of petechiæ is counted. If many are present the test is repeated not less than forty minutes later on the other arm at a lower pressure of 35 mm. of mercury. The results are graded according to the following scheme:—

- Grade I. No petechiæ within the examined skin area at 50 mm. pressure for fifteen minutes.
- Grade II. Petechiæ appearing at 50 mm. mercury, but less than six in number.
- Grade III. More than six petechiæ at 50 mm. mercury, but two or less at 35 mm. mercury.
- Grade IV. At least two petechiæ at a pressure of 35 mm. mercury.

Göthlin [1048] emphasizes that the pressure of the cuff must be infra-diastolic so that there is an uninterrupted flow of blood to the forearm.

The test is carried out at room temperature (16°–21° C.) and the patient should not have a hot bath on the day of the test or take any exercise within three hours of it. Göthlin and his pupils have done a considerable amount of work on vitamin C deficiency in Scandinavia, using this test. He considers that more than six petechiæ at 50 mm. pressure for fifteen minutes are abnormal and he believes that this capillary fragility test serves as a measure of the physiologically indispensable minimum requirement for vitamin C. From simultaneous vitamin C tests on blood a positive Göthlin test is stated to correspond to a blood level of between 0.1 and 0.14 mg. of vitamin C per 100 c.

Wright [522] has introduced a modification of the Göthlin test. He inflates the pressure cuff midway between the systolic and diastolic pressures for fifteen minutes and then counts the petechiæ in a 2.5 cm. circle on the flexor surface of the forearm. A petechiæ count of 0 to 10 is considered normal; from 10 to 12 borderline; and above 12 abnormal.

Dalldorf [528] employs a *negative pressure method* for determining capillary fragility. A 1 cm. suction cup is applied to the skin of the upper arm near the deltoid insertion and varying pressures applied for one minute. If petechiæ appear at a negative pressure of 25 mm. of mercury or below, the capillary fragility is considered abnormal.

Scarborough [510] determines the capillary resistance in three standard areas on the arm, using negative pressure which is applied for half a minute. Lløystrup [1084] uses a suction cup and notes the lowest pressure at which petechiæ begin to appear in the whole area under the cup. He claims that there is a close correlation between capillary fragility and the serum vitamin C level.

The capillary fragility test has been widely used, but the results obtained are not consistent. Bell [524] and his colleagues observe the petechiæ in a circle 60 mm. in diameter. The pressure employed is 50 mm. of mercury for fifteen minutes, and the petechiæ are counted under the illumination from a 800 watt lamp 2 feet above the arm. Under these

conditions a petechial count of eight or less is considered normal. Khan and Minn [525] using the same method found that well-nourished middle-class children showed normal capillary resistance, while from twenty-five to thirty-three per cent. of children from poor quarters, and presumably receiving less vitamin C, showed increased capillary fragility. This was restored to normal levels by giving supplements of vitamin C.

On the other hand, several investigators have pointed out the unreliability of the test. Green [526] was among the first to show that it may give false positive readings in persons not suffering from vitamin C deficiency. Fox [347], Weld [527] and Crandon [68] have failed to obtain positive readings even in cases of frank scurvy, and Schultzer [369] states that he was unable to obtain a reduction in the number of petechiæ in a case of scurvy treated with vitamin C. Heinemann [558] even noticed an increased number of petechiæ in some cases after saturating them with vitamin C. He further observed that of seventeen nurses living under identical conditions of housing and nutrition, eight developed less than five petechiæ, five developed more than ten, and four had numerous petechiæ in response to the capillary fragility test. Yet all were of the same age, in good health, and not menstruating at the time of the test. This is confirmed by the observation of Scarborough and Gilchrist [1002], who found no correlation between capillary fragility and plasma vitamin C levels. Actually they found that the capillary fragility increased with a rising blood vitamin C.

Other investigators have also found little or no agreement between the capillary fragility test and blood vitamin C studies [528-530]. Rapaport and his colleagues [350] studied a hundred and fifty children at the Mount Sinai Hospital, but could find no correlation between abnormal capillary fragility and avitaminosis C as tested by other methods. They also report that the administration of orange juice to scorbutic infants did not produce any constant effect on the capillary fragility [531]. The method is also criticized by Difs [532], who found variations in tests made on the right and left arms at the same time, and on the same subject at intervals of fourteen days.

Bell and his co-workers [369] determined the capillary fragility of a group of 346 normal students between 1937 and 1939, and again during the war year of 1942; there was no significant difference in the petechial distribution curves of the two groups in the different periods, in spite of the reduced intake of vitamin C in the 1942 group as a result of the war (see Widdowson and Alington, Harris, Francis and Wormall, p. 505).

Bell, Lazarus, Munro and Scarborough [507] have shown that although the positive and negative-pressure methods give results that are consistent within themselves, the results from the two methods are not comparable.

Beaser, Rudy and Seligman [1010], in the course of investigations on diabetic retinitis, noted many instances of increased capillary fragility in the absence of blood dyscrasia or obvious vitamin C deficiency.

It is clear from these observations that capillary fragility cannot be employed as an index of vitamin C nutrition. Capillary fragility varies with age, season, the time of the day, thickness of the skin and its degree of vascularization, and the part of the body on which the test is employed.

False positive results are liable to be obtained during menstruation and after hot baths, in septic and acute infectious conditions, *e.g.*, diphtheria, scarlet fever, in thrombocytopenic purpura, anæmia, hæmophilia, reactions to arsphenamine and related drugs, acute nephritis, the terminal phases of malignant disease, allergic states and malignant hypertension. Capillary fragility is most probably not a function of vitamin C nutrition. The work of Scarborough suggests that vitamin P is concerned with its control (p. 861). Only a third of all cases of scurvy show an increased capillary fragility [1088].

The Intradermal Test. In 1937 Rotter [533] described a test for the diagnosis of vitamin C deficiency depending upon the power of the vitamin C of the skin to decolorize the blue dye, 2 : 6-dichlorophenolindophenol. His test consists in injecting 0.1 c.c. of a 1 in 400 dilution of the dye into the skin of the volar surface of the forearm. According to Rotter, if the individual is saturated the vitamin C in the skin decolorizes the dye in less than five minutes; normal cases decolorize it in five to ten minutes, and unsaturated individuals require longer than ten minutes. Portnoy and Wilkinson [534] studied the test on a hundred and three patients, and on the whole were inclined to accept its validity. They considered that it might be of considerable value as a rapid diagnostic test for vitamin C deficiency. Beck and Krieger [535] and Banerjee [1072] also believe that the test is quite adequate for rough clinical purposes, although they admit that it is not as accurate as blood or urine tests. They consider that vitamin C deficiency exists if decolorization takes more than thirteen minutes. Slobody [1026], who injects about 0.05 c.c. of N/300 of the dye solution, states that decolorization takes more than fourteen minutes if the plasma vitamin C level is below 0.8 mg. per cent. This, he says, represents a definite degree of unsaturation; a decolorization time of nine to thirteen minutes indicates mild deficiency; and nine minutes or less indicates an adequate degree of vitamin C nutrition. The test has been employed by Dollé [864]; Reddy and Sastry [841], and by Uzan and Bronstein [536], who have modified it by using methylene blue as the dye.

A number of other workers have submitted the test to extensive trial, but, with the exception of Slobody [1026], they have found no correlation between the decolorization time of Rotter and the clinical condition of the patient, or between it and vitamin C studies on blood and urinary excretion [537-545]. Technically it is difficult to inject small quantities of solution with precision, but even using an improved syringe capable of injecting exactly 0.01 c.c., Goldsmith and Ogaard [542] and Poulsen and Lieck [543] could obtain no correlation between the intradermal test and blood serum values. Changes in position and temperature considerably affected decolorization time. Goldsmith subjected the test to a statistical study and her results indicated no specificity for the method. MacLenathen [541] found that the variations in the decolorization time were too great for any normal standard to be set up, and there was frequently a wide variation in the response at areas within a few centimetres of one another. On the other hand, Banerjee and Guha [546] think that the intradermal test might give a more accurate account of the state of vitamin C nutrition than urinary saturation tests (p. 544). Other factors that have not been

considered are the possibility of other reducing substances in the skin, and local changes in the circulation, oxygen supply, and lymphatic drainage of the forearm. With all these possible variables the test does not appear to have any clinical value.

Urinary Excretion of Vitamin C. Single determinations of the urinary excretion of vitamin C are of no value whatsoever as an index of vitamin C nutrition. Some authorities, however, consider that information on the twenty-four hour excretion of the vitamin is reliable for the detection of vitamin C deficiency. Twenty-four hour collections of urine are preserved by adding enough twelve per cent. metaphosphoric acid to the receptacle to maintain a final concentration of two to three per cent. An alternative but less satisfactory method is to add 10 c.c. of glacial acetic acid to every 100 c.c. of urine, which is kept in a refrigerator or dark bottle. 8-Hydroxyquinoline is also a good preservative. Using acetic acid there is a loss of ten per cent. of vitamin C for every hour's storage; with phosphoric acid the loss in the same time is only one per cent. [1008]. The principle of the estimation is to take a measured amount of recently standardized 2 : 6-dichlorophenolindophenol and determine the amount of urine which has to be added to it to discharge its colour, the titration being carried out rapidly [547]. The dye is made up in aqueous solution at a strength of about 0.1 per cent., so that 0.05 c.c. (*i.e.*, 0.5 mg.) is equivalent to roughly 0.025 mg. of ascorbic acid. The solution should be freshly prepared and standardized against crystalline vitamin C, 0.025 gm. of which are dissolved in 50 c.c. of water for the purpose of standardization. The purity of the vitamin C is further checked against 0.01 N iodine solution. In titrating the urine a 2 c.cm. microburette reading to the nearest 0.01 c.cm. is used. The urine is run from the burette into the dye solution conveniently contained in a pointed centrifuge tube.

2 : 6 Dichlorophenolindophenol is also decolorized by other substances present in the urine, such as glutathione, cysteine, thiosulphates, and it has been stated that reducing substances other than vitamin C may account for up to thirty per cent. of reduced dye. Atropine, aspirin and pyridium [559] also interfere. Vitamin C does not reduce 2 : 6-dichlorophenolindophenol in the presence of twenty per cent. hydrochloric acid [985]. This affords a method of estimating in the presence of interfering reducing substances. Richter and Croft [1008] estimate the "true" vitamin C in urine by eliminating thiosulphates and sulphur-containing substances in the urine with lead acetate before titration with indophenol dye. Reducing substances other than vitamin C can also be precipitated with phosphotungstic acid [557]. The specificity of the indophenol method is increased by adding formaldehyde [889].

Vitamin C can be estimated in urine by an entirely specific method depending on its oxidation to dehydroascorbic acid, which is separated as the crystalline 2 : 4-dinitrophenylhydrazine derivative [860]. This is then converted into furfural, which is estimated colorimetrically with aniline acetate. Meiklejohn and Stewart [844] have also devised a method for the specific estimation of vitamin C in urine, depending upon its oxidation by ascorbic acid oxidase, a most convenient source of which is autolysed cucumber juice.

In the early work of Harris [547] and his associates the expression "resting level" was used to indicate the day by day excretion of the vitamin, with an excretion of 10 mg. representing the borderline between deficiency and adequacy, 20 mg. a moderately low intake, and 40 mg. a liberal intake of the vitamin. The common range of excretion given by others is 10 to 50 mg., although no definite standard is available [820, 548, 375, 380]. Magnusson and Osterberg [549] found that the daily excretion of normal subjects ranged from 10 to 80 mg. According to van Eekelen [316] a daily excretion of 40 mg. indicates that the subject is in a state of saturation; Spellberg and Keeton [554] give the minimum normal excretion as 20 mg. a day. The urinary excretion of vitamin C is not seriously influenced by diuresis [550].

The value of simple excretion tests as a measure of vitamin C deficiency is very doubtful. Thus Kellie and Zilva [359] have shown that the amount excreted can be made to vary at will by adjustments of the diet. Kassan and Roe [367] examined a series of apparently normal medical students and found that fourteen excreted no vitamin C, yet they had no signs or symptoms of scurvy, either frank or subclinical. Similarly, Roe and Hall [360] state that no less than fourteen out of fifty subjects on an apparently adequate diet showed no excretion of vitamin C. Pijoan [1075] lived on a diet poor in vitamin C and excreted none in the urine for twenty months without any ill effect. Fox [344] has repeatedly observed low excretions for African natives in good health. Conversely the urinary excretion of vitamin C is not always as low as would be expected in cases of frank avitaminosis. Schultzer [369], for example, found mean daily excretions in scorbutic patients ranging from 13 to 24 mg. According to some observers a daily excretion of 24 mg. of vitamin C would indicate an adequate intake of the vitamin.

It must be remembered that there are several factors, including a fluctuating renal threshold, which may affect the urinary excretion of vitamin C besides a diminished intake (p. 502). Deeny [970] has shown that marked hourly variations occur in the excretion of vitamin C on a standard intake, and at all physiological levels of vitamin C.

Twenty-four-hour specimens are difficult to secure without loss from ambulatory patients or indeed any but the most co-operative hospital patients. According to Wright [555] if urine is stored for twenty-four hours, instead of being titrated immediately on voiding, losses in the region of twelve per cent. of the vitamin C may occur. Holmes and Campbell [556] have observed losses as great as forty-six per cent. in partially filled bottles in warm weather. They state that the urine can be preserved for forty-eight hours if kept in a dark bottle with 50 c.c. of glacial acetic acid, 3 grams of calcium carbonate and a 2 gram fragment of marble. This decomposition can be largely prevented using phosphoric acid as a preservative [1008].

Saturation Tests (Test Dose). The urinary excretion of vitamin C following the administration of a test dose of the vitamin has been used as a criterion of the vitamin C saturation of the body and as a means of detecting early states of vitamin C deficiency. The rationale of the test is based on the hypothesis that, following the administration of vitamin C,

the requirements of the tissues for the vitamin are satisfied before the concentration of vitamin C in the blood rises to the threshold value and is eliminated in the urine. If a test dose of vitamin C is given to a normal subject receiving an adequate intake of the vitamin (25 to 50 mg. daily) a large rise in the urinary excretion of vitamin C occurs generally on the first day or certainly on the second; if the vitamin C intake is lower than normal the response is less or delayed by one or more days (Harris [560]). Criticisms of the saturation method have been dealt with by Harris [866].

Oral Test Dose. According to the original technique of Harris and Ray (1935) twenty-four-hour specimens of urine were collected on consecutive days to determine the "resting level" of excretion and the response after repeated daily test doses [885]. In 1937 Harris and Abbasy [856b] introduced a simplified technique circumventing the need for a full day's specimen, and suitable for use in schools, hospitals and institutions. The following hours only give the time relationships and can be altered to suit individual requirements.

At 9 a.m. the individual to be examined empties the bladder and the specimen is rejected. Another specimen is obtained at 12 a.m. and titrated for vitamin C; any urine passed during the three hours is also collected and titrated. The same programme is repeated on a second and, or, third day. After this, for one or two further days, or more if necessary, the standardized test dose is given at 10 a.m. and the urine collected on the same day between 2 and 4 p.m. or preferably 5 p.m. This two or three hours afternoon specimen reveals if there has been an adequate response to each day's test dose. According to Harris it coincides with the peak of excretion, *i.e.*, four to five hours after the administration of the test dose. Individuals whose past intake of vitamin C has been adequate, *i.e.*, of the order of 25 to 50 mg. per 10 stone of body weight, show a satisfactory response generally on the first day and certainly on the second day. With an increasing degree of deficiency there will be an increased number of days delay before saturation is reached and the excess vitamin flows over into the urine. The test dose employed by Harris is 700 mg. of vitamin C in 100 c.c. of water per 10 stone of body weight. The actual deficiency of vitamin C can be obtained by multiplying the daily test dose by the number of days elapsing between the beginning of the test and the sharp rise in the urinary excretion. Using this test Harris [560] reports a high incidence of vitamin C deficiency in many school children.

In a more recent paper Harris [1004] has correlated the daily intake of vitamin C with the number of days required to saturate the individual on a standard daily test dose of 700 mg. of vitamin C per 10 stone of body weight. He takes "saturation" to be an excretion of 50 mg. or more of vitamin C per 10 stone of body weight in a two and a quarter hour specimen collected about the fourth or fifth hour after the test dose. Harris states that with a daily intake of 45 to 75 mg. or more of vitamin C a response occurs on the first day of the test dose, *i.e.*, 50 mg. or more per 10 stone of body weight is excreted in a two and a quarter hour urine specimen. On a daily intake of 37 mg. of vitamin C the response occurs on the first to second day after test dosing; on 23 mg. daily a response occurs on the second to third day; in scorbutic subjects seven to ten days is required

for saturation. Persons receiving 80 mg. as recommended by the Technica Commission on Nutrition of the League of Nations (1939) would thus attain saturation on the first or second day of test dosing.

Beck and Schorlemmer [568] give a daily test dose of 300 mg. of vitamin C orally at 6 a.m., and collect the urine between 7 a.m. and 1 p.m., the bladder being, if possible, emptied only once, at 1 p.m. In normal persons the vitamin C excretion in the six-hour period is stated to be about 5 mg. per 100 c.c. of urine. This figure is also given by Gander and Niederberger [569] for a three to five-hour excretion period after a test dose of 300 mg. Considerable discussion has arisen over the period which should elapse before the urine specimens are taken. Richardson and Mayfield [561] question the validity of the six-hour period allowed by Harris and others between the taking of the test dose and the collection of the urine samples for titration. They find that the volumes of urine and the amount of vitamin C excreted at each urination differ considerably even in the same individual. When they compared the amounts of vitamin C excreted during six-hour periods, taken between 7 a.m. and 1 p.m., with the total amounts of vitamin C excreted during complete twenty-four-hour periods, they found that the variations in the results were so considerable that they doubted the value of a six-hour collection period for determining the "resting level" of vitamin C. They consider a twenty-four-hour specimen of urine essential.

On the other hand, Pemberton [562] has shown that the maximum increase in the urinary excretion of vitamin C occurs six hours after a test dose of 70 mg. per stone of body weight. He argues that there is no need to attach any importance to the actual value of the vitamin C excreted or to the volume of urine; all that matters is a sharp rise in the excretion within six hours of the test dose. If this occurs the previous level of vitamin C nutrition of the individual tested was adequate; if no sharp increase occurs some degree of deficiency can be diagnosed. In the case of children Pemberton gives a test dose of 50 mg. per stone of body weight. Using the Pemberton technique Wallace and Adler-Tanz [1074] state that only nine per cent. of children examined at the Royal Hospital for Sick Children, Glasgow, are saturated with respect to vitamin C; sixty-nine per cent. were unsaturated, and twenty-three showed what the authors call "suboptimal saturation."

There are several variations both in the size of the test dose and in the period allowed for collecting the urine. Engelfried [1052] states that a six-hours' test and a dose of 200 mg. is most satisfactory. Doses as low as 100 mg. and as high as 1,000 mg. have been used. Widenbauer [876], Jezler and Kapp [861], Levcowich and Batchelder [877] and Jezler and Niederberger [568] employ test doses of 200 to 500 mg. of vitamin C daily, and consider that the excretion of fifty per cent. or more of the doses within twenty-four hours or of the half-day dose within twelve hours is evidence of saturation. Spellberg and Keeton [554] find that thirty per cent. of a 600 mg. test dose is excreted in twenty-four hours. Similar figures are given by Youmans [565] and Wright [566], who considered an output of less than twenty per cent. a sign of suboptimal vitamin C nutrition. The amount excreted depends on the size of the test dose; the larger

the dose the lower the percentage excreted. This makes it difficult to compare observations of different investigators who use test doses of varying size. Baumann [564] believes that the degree of saturation of the tissues is best determined by oral administration of small doses of vitamin C daily for several days; the doses are of the order of 50 mg. for small children and 100 mg. for older children and adults. Under such conditions normal persons excrete from sixty to eighty per cent. of the test dose on the third or fourth day.

Goldsmith and Ellinger [567] examine the state of vitamin C nutrition by measuring the urinary excretion of vitamin C for six hours after the administration of a test dose of 600 mg. of the vitamin. A peak in the excretion is reached sometime between the first and sixth hour in a person on an adequate intake of vitamin C; this is followed by a gradual decline, approaching the original level at the end of twenty-four hours. If there is no rise in the urinary excretion of vitamin C after the test dose the level of vitamin C nutrition is considered inadequate. Goldsmith and Ellinger consider that the individual is normal if the urinary excretion of vitamin C over the six-hour period is 50 mg. or more; if over 100 mg. is excreted, partial or actual saturation of the tissues is assumed. Less than 50 mg. suggests depletion of the vitamin C stores. Between the third and the sixth hour forty-four per cent. of the test dose is excreted on an average. Goldsmith and Ellinger [567] have also devised a blood test for estimating vitamin C deficiency (p. 554). They report considerable vitamin C deficiency as a result of the routine examination of patients in hospitals and clinics. In one group fourteen out of twenty-two patients were found to be suffering from vitamin C deficiency, as shown by chemical tests, yet they appeared quite normal clinically.

Stotz and his colleagues [842] have developed an oral vitamin C tolerance test depending on the fact that if the tissue vitamin C is low, then on administering the vitamin orally it should flow from the plasma to the depleted tissues; if the tissues are saturated then the oral administration of vitamin C should result in a marked rise of plasma vitamin C. The test is best performed in the morning. A sample of blood is taken from the patient for the initial vitamin C content, and a dose of 6 mg. of vitamin C per kilogram of body weight given orally in water. Blood samples are taken two and a half and five hours later and the vitamin C content determined. No food other than coffee and toast is allowed. Blood vitamin C values in milligrams per cent. are plotted against time. Saturated patients and those receiving an adequate intake of vitamin C show a high initial vitamin C level in the blood, a marked rise after two and a half hours and a slow fall. The rise signifies that the rate of intestinal absorption of vitamin C exceeds the rate at which the relatively saturated tissues absorb the vitamin from the plasma. In patients suffering from vitamin C deficiency there is a low initial vitamin C level in the blood and only a slight or transitory rise, or even no rise at all, in the tolerance curve.

Parenteral Test Dose. Some investigators prefer to give the test dose of vitamin C intravenously or subcutaneously, because of the possibility of uncertain absorption. It has been shown, however, that the bulk of

an oral dose of vitamin C is absorbed from the intestine and excreted *viâ* the kidney unless the patient is suffering from a gastro-intestinal condition liable to interfere with absorption (p. 498). Parenteral administration of the test dose is therefore unnecessary in most cases. Wright [570] gives a test dose of 1,000 mg. intravenously and measures the subsequent urinary excretion of the vitamin over a five-hour period. He states that normally 500 mg. of this test dose would be excreted in twenty-four hours, and eighty per cent. of this, *i.e.*, 400 mg., within five hours of administering the test dose. Similar tests have been advocated using smaller doses of from 100 to 300 mg. For example, Ralli [572] and her co-workers employ a test dose of 100 mg. given intravenously, and regard an excretion of 40 mg. during the first three hours as evidence of normal saturation; values of the order of 11 mg. or less indicate deficiency.

Kastlin and Schlesinger [578] detect vitamin C deficiency by giving a test dose of 500 mg. intravenously, with observations on the blood level and urinary excretion over a four-hour period, which they claim is proportional to the excretion twenty-four hours after the test dose. Forty per cent. or more of the test dose is excreted within four hours in a normal person.

The intravenous administration of test doses of more than 800 mg. of vitamin C is criticized by van Eekelen and Heinemann [574], who argue that this causes the blood stream to be temporarily flooded with vitamin C, which flows over into the urine even in a patient with vitamin C deficiency, thereby simulating saturation. Erroneous conclusions may thus be drawn. They therefore give a test dose of vitamin C not exceeding 800 mg., or 4 mg. per kilogram of body weight. This is given subcutaneously, and the whole of it is said to be excreted within six hours with the peak excretion occurring before the end of the third hour. Berryman and others [1053] have shown that after the intravenous injection of 200 mg. of vitamin C to subjects receiving 100 mg. in their diet there is a considerable variation in the amount of vitamin excreted.

Ramel and Schenk [611] give fasting subjects 500 c.c. of tea and an injection of 200 mg. of vitamin C and measure the excretion in the following three hours. They consider an excretion of less than 18 to 20 mg. indicates deficiency.

Several investigators [320, 550, 576] have pointed out that in saturation tests a delay in excretion may occur in persons with impaired renal function (see p. 502). Ludden and Wright [550] state that erroneous values may be observed in such persons when the vitamin C content of single urine specimens obtained three, five, six or eight hours after any test dose, of whatever size, are used as criteria of the state of vitamin C nutrition. They agree that in the absence of renal impairment the estimation of the urinary output of the vitamin over a five-hour period after a dose of 1 gram gives satisfactory results. Ludden and Wright have introduced a modified technique, which they say is valid even in cases of impaired renal function, with the exception of uræmia. The test is conducted as follows :—

The patient omits breakfast on the morning of the test. Immediately after he has micturated and discarded the preliminary specimen of urine, 1 gram of vitamin C in 10 c.c. of normal saline is given intravenously. A

specimen of urine is collected exactly one and a half hours after the injection and another exactly five hours after. The twenty-four-hour output can then be predicted from the formula, $\frac{ab}{1.26a - 0.27b}$, where a and b are the vitamin C excretions over one and a half and five hours respectively. Wright and Ludden term this expression the *Saturation index*. The formula is arrived at from a definite correlation between the percentage of the first five hours' excretion of vitamin C excreted during the first hour and a half, and the percentage of the twenty-four hours' excretion excreted during the first five hours. This correlation is said to hold over a wide range.

Criticism of Test Dose Studies. In criticism of the test dose technique for detecting vitamin C deficiency it must be admitted that it has not yet been demonstrated that "saturation" with vitamin C is a normal state; in fact it is definitely abnormal because very few subjects are saturated, with respect to vitamin C if they rely on natural sources of the vitamin. Further, many people in the best of health have been shown to be unsaturated with respect to vitamin C. As Crandon [68] has shown, it is possible to feel in good health and excrete no vitamin C. At the moment we do not know at what stage "unsaturation" may begin to have a deleterious effect on health.

The measurement of the level of vitamin C nutrition by the test dose technique is a purely arbitrary procedure. A massive and unphysiological dose of vitamin C is given and the excretion studied over a period of several hours, the dose varying from one worker to another. The interpretation of the results also depends upon the individual worker. Some give daily supplements until there is a sudden rise in the urinary excretion; others consider that the subject is saturated when fifty per cent. of a test dose is excreted. If these arbitrary figures are accepted, and there is no reason why they should be, there are still sources of error. The renal threshold varies in different persons [881, 882]; the excretion of vitamin C also depends upon the acid-base equilibrium and the composition of the diet; and Deeny [970] has shown that the excretion of vitamin C on a given intake varies markedly from hour to hour, thus throwing considerable doubt on the value of the test dose technique in which the excretion of vitamin C is found during a period of four to six hours after giving the test dose.

Estimation of Vitamin C in the Blood. The 2:6-dichlorophenolindophenol titration test has been applied to the estimation of vitamin C in blood. A number of techniques using this test have been developed for the estimation of vitamin C in the plasma [577-582]. Oxalated blood is first centrifuged and the plasma deproteinized with metaphosphoric acid. After recentrifuging, the clear liquid is titrated against the dye indicator as for urine (p. 548). A method has also been developed, using methylene blue as indicator [588, 584], and the accuracy has been improved by the use of the photo-electric cell [585-587]. Using a methylene blue micro-method Butler and Cushman [1006] estimate the vitamin C in 0.2 c.c. of capillary blood. Actually isolated analyses of blood plasma values have proved quite worthless in estimating degrees of vitamin C subnutrition.

Estimation of the plasma vitamin C is only a measure of the immediate nutritive or metabolic level and is dependent on the recent dietary intake.

High fasting values may indicate satisfactory nutrition [466], but low values do not provide a reliable index. There is some evidence that the threshold value may vary in different individuals [881, 882, 595], and that plasma values are higher during menstruation [1007]. Some observers consider that plasma vitamin C values below 0.5 to 0.7 mg. per cent. indicate a state of vitamin C depletion and that values from 0.7 to 1.0 mg. indicate mild deficiency. These figures are purely arbitrary ones based on "saturation" tests and there is no clinical justification for them. Ralli [878] and her associates believe "saturation" to be the ideal, with blood plasma values of 1 mg. per cent. or over. According to Bryan [466] and his co-workers saturation occurs with intakes of 1.7 to 1.9 mg. of vitamin per kilo of body weight. It has never been satisfactorily demonstrated that the body is saturated with respect to vitamin C in normal healthy persons.

Thysell [1079] gives the following relationship between the intake of vitamin C and the plasma vitamin C level :—

Daily intake in mg.	Vitamin C in plasma mg. per 100 c.c.
100 or more	1.0-2.0
50-100	0.4-0.8
30-50	0.2-0.6
15-30	0.0-0.4
Less than 15	0.0-0.2

Brown and co-workers [1009] estimated the plasma vitamin C of over 800 men and women students in America. They found that the level in the men was lower than in the women, and lower in those who had meals out in cafeterias or restaurants than in those who lived at home. Their results are given below :—

Plasma Vitamin C. in mg. per 100 c.c.	Per cent. Women.	Per cent. Men.
1.0 or more .	28.8	7.8
0.8-0.99 . .	26.8	16.4
0.4-0.79 . .	42.8	49.4
Less than 0.4 .	7.6	29.6

They consider that 0.4 mg. is a scorbutic level, although not indicating clinical scurvy; 0.4 to 0.8 mg. indicates a definite deficiency; 0.8 to 0.99 mg. represents a "good normal," and 1.0 mg. or over indicates saturation. According to McMillan and Inglis [1041] there is no relationship between the blood figures and the clinical picture in scurvy.

On a daily intake of 80 to 80 mg. of vitamin C, which covers the

recommended intake of the National Research Council, U.S.A., the plasma vitamin C is about 0.7 mg. per 100 c.c. The general opinion is that a fasting plasma vitamin C level of 0.6 to 0.8 mg. or more is adequate, although this range is purely arbitrary and has no justification. The variations in the plasma vitamin C levels that have been found will be seen in the following table:—

Vitamin C in Blood Plasma

Author.	Subjects.	Number of Cases.	Mean mg./100 c.c.	Range mg./100 c.c.
Farmer and Abt [579] .	Medical students .	6	1.24	0.69-2.8
Taylor <i>et al.</i> [578] .	"	33	1.61	0.83-2.43
Greenberg <i>et al.</i> [580] .	"	55	0.72	0.25-1.48
Wright <i>et al.</i> [571] .	Hospital patients .	49	0.62	0.27-0.68
Pi Joan and Klemperer [581].	Normal	150	—	0.65-2.00
Rao [601]	Gastric ulcer patients	15	0.90	0.41-1.92
Braestrup [389, 602] .	Women in childbirth	22	0.26	0.08-0.72
	Patients	—	0.25	0.10-0.68
	Citrus fruits twice weekly.	—	0.86	0.16-0.64
	100 mg. vitamin C daily.	—	0.86	0.48-1.02
Wortis <i>et al.</i> [296] .	Subnormal . . .	68		0.4 or less
	Intermediate . .	35		0.4-0.69
	Normal	30		0.7 or over
Portnoy and Wilkinson [600].	High vitamin C diet	10	1.21	0.60-1.85
Kaiser and Slavin [603]	Tonsillitis . . .	123	0.79	0.53-1.08
Jetter [538] . . .	Normal	7	—	1.10 or over
	Hypovitaminosis C .	43	—	0.40-1.10
Snelling and Jackson [397].	Pregnant women up to 5 months.	12	0.6	—
	Pregnant women up to 8 months.	10	0.43	—
Goldsmith and Ellinger [567].	"Deficient" . . .	14	0.17	0.04-0.52
	"Normal"	11	1.11	0.48-1.98
	Subnormal	26	—	0.00-0.69
	Normal	42	—	0.70-1.29
	Saturated	32	—	1.30-1.99
Abt, Chinn and Farmer [473].	"Adequate" intake of vitamin C.	15	0.87	0.64-1.20
	"Inadequate" intake of vitamin C.	9	0.64	0.44-0.92
Croft and Snorf [521] .	Student nurses in good health.	50	0.81	0.60-1.12
	Hospital patients .	38	less than 0.40	
Lever and Talbott [591]	Healthy normals receiving adequate vitamin C.	68	0.6	0.10-1.46
Kassan and Roe [367] .	Medical students .	50	0.76	0.13-1.68
	Prison inmates .	50	0.16	0.02-1.13
Fincke and Landquist [977].	College students .	5	0.8	—

Vitamin C in Blood Plasma—continued

Author.	Subjects.	Number of Cases.	Mean mg./100 c.c.	Range mg./100 c.c.
Butler and Cushman [590]	"Normal" . . .	10	0.6	0.8-2.5
Secher [668]	Scurbutic patients . "Normal" and hospital patients.	7 ?	0 —	— 0.1-1.2
Prunty and Vass [1005]	Saturated "normal." "Average" . . . Students . . . Hospital patients .	— — — —	0.8 0.56 0.4 —	— — — 0.12-0.24
Lewis, Storvick and Hauck [976].	"Normal" on 75 mg. vitamin C daily.	6	1.1	1.0-1.2
Storvick and Hauck [878]	"Normal" on 50 mg. vitamin C daily. On 200 mg. vitamin C daily.	2 2	0.85 —	— 1.07-1.58
Dodds and MacLeod [997]	85 mg. intake daily 60 mg. " " 85 mg. " " 110 mg. " "	12 12 12 12	— — — —	0.27-0.76 0.44-1.25 0.5-1.2 0.6-1.4

Crandon [68], Fox [844], Rietschel [342] and others have shown that plasma vitamin C levels may be exceptionally low without clinical evidence of vitamin C deficiency. According to Rietschel plasma levels of 0.2 to 0.8 mg. are not abnormal. He kept a subject on a diet free of vitamin C for 100 days without producing scurvy; the vitamin C plasma level was zero. Pijoan [1075] also maintained a plasma vitamin C level of 0.0 to 0.2 mg. per cent. for twenty months without ill effects. Dagulf [379] also reports that three hundred and twenty-six healthy Swedish people had an average plasma vitamin C of only 0.22 mg. per 100 c.c. In scurvy, too, the values are not always exceptionally low, though they all fall within the range of 0.0 to 0.5 mg. There is certainly complete lack of correlation between the clinical condition and plasma vitamin C values. Thus Croft and Snorf [521] noted blood values of only 0.12 mg., and Kassan and Roe [367] levels of 0.02 mg., without any signs of scurvy. Owens [257] has also observed levels below 0.4 mg. in fifty diabetics without clinical evidence of vitamin C deficiency. Holmes [637] examined the plasma vitamin C level in sixty apparently healthy children and low levels were observed, which persisted over a period of ten months. The ability to maintain a fixed level of vitamin C in the plasma, no matter how small, indicates a positive vitamin C balance [1075].

On the other hand, there is evidence that accurate information on the level of vitamin C nutrition can be obtained from analyses of whole blood [801, 858, 367, 882] or of the white cells and platelets [588-590]. Butler and Cushman [590] have shown that whole blood, and the white layer of the centrifuged blood of subjects whose vitamin C nutrition is relatively poor may contain measurable amounts of vitamin C (25 to 38 mg. per 100 gm.), even when the plasma level is zero. Crandon [68], whilst

on a scurvy producing diet, noted that after forty-one days his plasma vitamin C was zero, but even after eighty-two days the white cell-platelet vitamin C was 4 mg. per 100 c.c. It would appear that the apparent vitamin C content of whole blood or of the white blood cells plus platelets of individuals not suffering from infection or leukæmia provides a far more reliable index of vitamin C deficiency than plasma values. Whole blood determinations afford the best index for "saturation." What is important is the tissue reserves of vitamin C (not the plasma level), which is best measured by the concentration of vitamin C in whole blood or the white cell layer. If this falls steadily vitamin C deficiency may be diagnosed. According to Butler and Cushman [590] the average normal vitamin C of the white cells and platelets is 34 mg. per 100 grams, with a range of 29 to 43 mg. They have shown that vitamin C passes from the plasma to the red blood cells and that the distribution ratio of the plasma concentration to the red cells concentration varies with the state of vitamin C nutrition. They have identified a reducing substance in the buffy layer of the blood with vitamin C, and believe that the reducing power of the buffy layer provides an index of physiologically significant vitamin C deficiency. In patients with leukæmia the concentration of reducing substance in the buffy layer was as high as 140 mg. per 100 grams when the plasma vitamin C was as low as 0.2 mg. If the plasma level of vitamin C were taken as an index of vitamin C nutrition, these patients would have been considered to be suffering from considerable vitamin C deficiency.

The Butler and Cushman method for estimating vitamin C in whole blood has been modified by Deeny, Murdock and Rogan [714] who saturate the blood with carbon monoxide before hæmolysis. This retards the oxidation of the vitamin C. Vitamin C can also be estimated in whole blood or the white cell layer by oxidizing it to dehydroascorbic acid, the 2:4-dinitrophenylhydrazine derivative of which is then estimated colorimetrically [360, 1086].

The plasma values do not reflect the amount of vitamin C stored in the body; they merely represent an overflow, and when this is high enough vitamin C is excreted. Whole blood values indicate the extent of saturation down to a state of marked depletion, and while values above 5 mg. exclude scurvy, values below this are equivocal. Farmer [1078] observed zero plasma vitamin C values in volunteers on scorbutic diets without clinical evidence of scurvy. It took seventy days on the diet for the plasma vitamin C to fall to zero. White blood cell concentrations of vitamin C are the best indication of scurvy. Technical difficulties attached to estimating the vitamin C in white cells have prevented its routine use.

Other blood tests for vitamin C deficiency have been used, in which a test dose of vitamin C is injected intravenously or intramuscularly and the blood levels of the vitamin studied at varying intervals afterwards. Kajdi [592] and her co-workers have proposed a new saturation test, in which the plasma vitamin C is determined before and four hours after the injection of 200 mg. of vitamin C. From these figures is calculated the *vitamin-C index*, which is defined as the initial plasma value multiplied by the increase in the plasma four hours after the injection of the vitamin multiplied by 100. Kajdi takes a four-hour interval because she finds that

the vitamin C blood plasma level reaches a constant value by that time. The test dose of 200 mg. was chosen because smaller amounts did not sharply illustrate the difference between depleted and partially depleted stores of vitamin C, and larger doses were no more accurate. Kajdi states that a vitamin C index below 0.8 indicates clinical scurvy; indices between 0.8 and 6 show a low vitamin C reserve; and an index of 10 or over corresponds to optimal vitamin C nutrition. It is claimed that in sixty-four out of seventy cases correct information on the vitamin C status of the patient was obtained, laboratory findings being checked against the condition found on clinical examination. Elmby and With [584] employ a saturation test of 500 mg. of vitamin C and determine the plasma vitamin C before the injection and eight times within the following four hours. This seems an unnecessary elaboration with much inconvenience to the patient.

Goldsmith and Ellinger [567] examine the plasma vitamin C in the fasting patient and again one and three hours after the administration of 600 mg. of vitamin C. They consider that the vitamin C status is adequate if the original level is over 0.7 mg. per 100 c.c. rising to 2 mg. or more three hours after the test dose. Similar tests have been employed using smaller intravenous test doses (100 to 300 mg.) [598]. Goth [594] detects vitamin C deficiency by injecting 300 mg. of the vitamin intravenously and examining the blood two hours later. If there is no rise in the blood level or only a slight one it is considered to indicate hypovitaminosis; a rise of at least 0.50 mg. is stated to occur if the individual is saturated.

A method of estimating vitamin C deficiency based on a test dose of 500 mg. of vitamin C intravenously with observations on both the blood level and urinary excretion over a four-hour period is described by Kastlin and his collaborators [578]. They claim that the urinary excretion after four hours is proportional to the urinary excretion in a twenty-four-hour period. The vitamin C is first estimated in a blood sample and specimen of urine after fasting for twelve to fifteen hours, and then 500 mg. of vitamin C is given intravenously. A blood specimen from the opposite arm is collected five minutes later. Subsequent blood and urine specimens are obtained one, two, three and four hours later. Then the blood vitamin C in milligrams per cent. and the vitamin C excreted in milligrams are plotted against time. The total urinary excretion in milligrams and the percentage of the test dose excreted are computed. Kastlin and his fellow-workers state that a typical curve of a "saturated" person shows the following characteristics: (a) The fasting blood level of vitamin C is 0.7 mg. per cent. or higher. (b) The five-minute period is relatively high—from 4.5 to 9 mg. per cent. (c) The rate of return of the blood concentration from the five-minute peak to the four-hour level is gradual, showing that the tissues have no great avidity for vitamin C. (d) The four-hour blood level is well above the fasting level. (e) Urinary excretion of the test dose is greatest in the first hour and the total urinary excretion in four hours is forty per cent. or more of the test dose.

The typical severe vitamin C deficiency curve is stated to show a marked deviation from the normal. (a) The fasting blood level of vitamin C is below 0.4 mg. per cent. (b) There is only a slight variable rise in the blood level after five minutes. (c) The blood concentration falls

rapidly to near the fasting level. (d) The total urinary excretion ranges from a few milligrams to twenty per cent. of the test dose. The authors of this test state that it may show evidence of severe vitamin C deficiency when there are no clinical signs of scurvy.

Rinehart and Greenberg [785] read the vitamin C plasma levels three and five hours after an oral dose of 15 mg. per kilogram of body weight. For the sake of simplicity they classify the resulting blood plasma curve as flat (peak below 0.5 mg. per cent.), medium (peak 0.5 to 0.9 mg. per cent.) or high (peak above 0.9 mg. per cent.). According to Rinehart and Greenberg the flat curves reflect tissue depletion of vitamin C and the medium curves moderate depletion.

Vitamin C in Cerebrospinal Fluid. Rohmer, Bezssonoff and Sacrez [1011] state that the level of vitamin C nutrition can be followed by estimating the vitamin C in cerebrospinal fluid. They give 5.5 to 7.5 mg. per litre as the normal range. There is no parallelism between the cerebrospinal fluid levels and the urinary excretion. Owing to the greater trouble of obtaining cerebrospinal fluid, the necessity for the patient to remain in bed after spinal puncture, and all the risks attending the removal of cerebrospinal fluid, the routine estimation of vitamin C in the latter has little to recommend it.

VITAMIN C IN THERAPEUTICS

The only disease that specifically responds to vitamin C is scurvy, yet its use has been advocated in the treatment of a number of conditions. A vitamin C deficiency has been detected in patients suffering from a variety of diseases, and while it may be rational to remedy this deficiency, particularly in infectious and surgical conditions, it is too much to expect the vitamin C to have any direct effect on the course of the disease. It is extremely difficult to tell whether the administration of vitamin C produces any clinical improvement unless a large number of cases is studied and compared with control cases not receiving the vitamin, but otherwise living under the same conditions and receiving the same treatment, food and nursing. Many of the studies have not been controlled and have only been carried out on a few cases. This makes their evaluation difficult.

With regard to dosage and administration there is no need to give the vitamin parenterally, unless there is any reason to suspect deficient absorption (as in persistent vomiting, colitis, diarrhoea), since absorption from the intestine is practically complete even when very large doses are given (p. 498). In severe cases of scurvy relief may be more rapid if it is given parenterally. Intravenous injection is not recommended as the renal threshold may be rapidly exceeded and a large part of the dose excreted in the urine. Intramuscular injection is therefore preferable. As a dietary supplement 100 mg. a day in divided doses is adequate, but when given therapeutically in infections and in cases where there is a severe deficiency a dosage of 500 to 1,000 mg. a day may be necessary. It should be taken several times a day, rather than in one large dose, with or just after meals, since the gastric acid aids absorption. Large doses should be given until one of the saturation tests (p. 544) shows that there

is a considerable rise in the excretion ; the dosage can then be reduced to a maintenance one of 100 mg. daily. Farmer [1078] states that even when the plasma vitamin C and the vitamin C in the white cell layer are zero an intake of 100 mg. daily will eventually produce tissue saturation.

Vitamin C in Infections. It may be repeated here that hospital and institution diets are notoriously deficient in vitamin C. Many diets do not provide more than 10 to 15 mg. daily, so that in calculating the requirements of say the surgical patient, or the patient with an infectious disease, the amount provided by the average hospital diet may be considered negligible.

It appears from laboratory and clinical studies that vitamin C plays a part in resistance to infection (pp. 476-481). It is, therefore, important to ensure a reasonable intake of vitamin C in patients suffering from infectious diseases. It may be a wise procedure to saturate all patients with pyrexia.

Diphtheria. The effect of vitamin C on the diphtheria toxin and the increased vitamin C requirements of patients suffering from the disease have been previously referred to (pp. 479, 522). Whether the administration of vitamin C as a therapeutic measure has any effect on the progress of the disease clinically is very doubtful. It is possible that low vitamin C reserves predispose to infection, although this is very difficult to prove statistically. Bamberger and Zell [604] treated a group of forty-one children suffering from toxic diphtheria with vitamin C and adrenal cortex extract, and found that the mortality was thirty-nine per cent. as against a much higher percentage in untreated cases. Szirmai [605] treated eighteen cases of toxic diphtheria with serum and vitamin C and had only one fatality ; in a control series of fifty-four cases not receiving vitamin C there were fifteen deaths. The better results in the first series were attributed to the combined effect of serum and vitamin C. Favourable results in some cases of toxic diphtheria are also reported by Otto [606], who gave vitamin C and adrenal cortex extract in addition to the usual serum treatment. Eleven out of twenty-six toxic cases showed definite improvement, but the vitamin C was of no value in the less severe form. Otto states that a definite therapeutic effect can be ascribed to vitamin C in arresting hæmorrhage in diphtheria, particularly epistaxis. The value of this observation is doubtful as there is little evidence that vitamin C controls hæmorrhage (p. 541).

Others have failed to confirm these observations. Thus Engelhard [607] selected fifty-five cases of severe diphtheria from a total of four hundred and seventy children. Alternate cases were given vitamin C and adrenal cortex extract, but the results were no better than in the control cases. Although they recommend the administration of vitamin C in cases of diphtheria to remedy the deficiency nearly always observed, Kündiger and Salus [185] admit that it has no specific action on the condition. Pakter and Schick [598] in America and Schwartzter and Stockebrand [608] found that the administration of vitamin C had no effect on the Schick reaction in children ; in all the positive cases tested the positive reaction was maintained undiminished. After two years' experience of treating

cases of severe toxic diphtheria with vitamin C, Dieckhoff [617] found that the vitamin had no favourable effect on the course of the disease.

Pneumonia. The work of Tonutti (p. 481) has shown that vitamin C may play an important part in the defence mechanisms against pneumonia, and a number of workers have shown that the excretion of vitamin C in pneumonia patients is very low. Gander and Niederberger [482] have attempted to correlate the seasonal incidence of vitamin C deficiency with that of pneumonia; according to them the peak mortality months for pneumonia coincide with those for the lowest level of vitamin C nutrition (about March). The figures of Anderson [1040] do not bear this out. His mortality figures for pneumonia in Glasgow show that the highest mortality is in January and the quarter preceding it. He states that the number of deaths in January is twice the average of the other eleven months. Undoubtedly nutritional deficiency plays a part in the ætiology of pneumonia, particularly in the very young. Glazebrook and Thomson [816] have shown that the incidence of pneumonia was lower in a large group of adolescent boys receiving supplements of vitamin C than in a control group that did not.

Gander and Niederberger [482] treated fifteen patients with vitamin C and recorded that considerable improvement occurred if the patients were saturated with vitamin C on the first day of the illness. Then the temperature dropped by crisis by the third day. A "remarkable improvement in the general condition" both early in the course of the disease and in convalescence was described. The scheme of dosage employed was 500 mg. intramuscularly and 300 mg. by mouth. During the next three hours 900 mg. of vitamin C by mouth was given. Some cases received 1,800 mg. a day. No controls were used in this study. Hochwald [609, 610] states that if 500 mg. of vitamin C is given intramuscularly every two hours an attack of lobar pneumonia can be cut short on the first day. If given on the second day the course of the disease can be shortened, whilst later treatment, although it does not arrest the course of the disease, lessens its severity. According to Hochwald [610] the improvement in general condition (prostration, dyspnoea), the fall in temperature, and improvement in the blood picture is particularly striking. Hochwald, Kalk and Frobenius [618] and Walther [614] consider that pneumonia is an allergic-hyperergic condition; a nasopharyngeal infection precedes the lobar pneumonia by two or three weeks and sensitizes the body. The anti-allergic effect of large doses of vitamin C has been demonstrated in animal experiments (p. 576) and Hochwald therefore justifies its use in pneumonia.

Other continental workers have stated that vitamin C in large doses, *e.g.*, 1 to 1.6 grams daily, produces clinical improvement in pneumonia [612, 615, 616]. These observations were made before the sulphonamides were in general use.

Tuberculosis. The diminished excretion of vitamin C in tuberculosis has been noted by a number of investigators [126-180]. Low blood vitamin C values have also been reported [683, 684, 849, 850]. Dagulf [618] concluded that the tuberculous patient requires about three times as much vitamin C as the normal person. As healing in tuberculosis is characterized largely by the formation of connective tissue, for which vitamin C is

essential, it does not seem unreasonable to suppose that vitamin C deficiency may delay healing and have an unfavourable effect on the course of the disease. On the other hand the administration of vitamin C to make good the deficiency, or even its administration in excess, may help to change the course of the disease from an unfavourable prognosis to a more favourable one. Getz and Koerner [849] state that an extreme degree of vitamin C deficiency appears to make the prognosis worse in tuberculous patients.

Animal experiments suggest that a vitamin C deficiency in animals predisposes to tuberculous infection. Thus Greene [619] and his co-workers found a shortened survival period and a decrease in the body weight of infected guinea-pigs on a scorbutic diet, and De Savitsch [620] found smaller lesions and a greater increase in weight in animals inoculated with tubercle bacilli and fed vitamin C than in the inoculated and untreated controls. Russell, Read and Rouse [1060] have shown that compared with controls there is a greater dissemination of tubercle bacilli in the viscera of infected scorbutic animals, and that the tuberculous lesions show more extensive caseation. They observed that the deposition of fibrous tissue around the tubercles was slightly greater in the organs of control animals receiving adequate vitamin C. These observations are in agreement with the early laboratory studies on scorbutic guinea-pigs, which often died of tuberculosis. McConkey and Smith [621] concluded that the administration of tuberculous sputum to guinea-pigs was not the sole cause of intestinal ulcers. Their control animals given adequate vitamin C developed ulcers in only two instances, compared with twenty-six in the group suffering from vitamin C deficiency. Steinbach and Klein [809] have claimed that the daily injection of vitamin C into tuberculous guinea-pigs increased their tolerance to repeated large doses of tuberculin. Similar observations were made by Birkhaug [150], who found that vitamin C definitely inhibits the tuberculin reaction in tuberculous guinea-pigs. He also records that large doses of the vitamin caused a significant increase in weight and reduction in the tuberculous lesions of guinea-pigs infected with tuberculosis. The histochemical studies of Tonutti and Wallraff [108] show that re-infection of experimental animals after a previous sensitization results in the accumulation of cells in the lung alveoli laden with vitamin C. In addition the bronchioles are often seen to be choked with desquamated cells saturated with vitamin C. Such studies explain why the vitamin C requirements are considerably increased in tuberculosis. Osborn and Gear [622] suggest that there is possibly a relation between the ability to synthesize vitamin C and the reaction to the tubercle bacillus, since tuberculosis chiefly occurs in those species unable to synthesize vitamin C (man, monkey, guinea-pig).

A number of studies have been made to see if large doses of vitamin C have any effect on the course of tuberculosis in human beings. Pelter [628] reported favourably on the treatment of forty-nine adults and twenty-four children with 150 mg. of vitamin C a day. There were, however, no controls and a preparation containing vitamin C, not the pure substance, was given. An extensive clinical study was made by Hasselbach [127, 624], who treated seventy patients suffering from various types of tuberculosis

with vitamin C. As a rule, 2,000 mg. of the vitamin was given weekly in divided doses of 800 mg. daily. A sharp fall in temperature was noted and treatment was particularly recommended in cases with small seeping hæmorrhages. Unfortunately, the study was complicated by the simultaneous administration of calcium and gold, so that it is difficult to assess the value of the vitamin C in the treatment. Patients who were stationary and with a good prognosis were said to benefit from the vitamin C, to which Hasselbach ascribed definite tonic properties. No controlled studies were reported. Sande [198] also recommends the use of vitamin C with gold therapy in cases with hæmorrhage. Melzer [625], although he advocates remedying vitamin C deficiency in tuberculosis, failed to observe that it had any beneficial effect in the treatment of hæmorrhage.

The vitamin C requirements of tuberculous patients were investigated by Heise and Martin [126], who found that between 55 and 140 mg. were required in active cases for the maintenance of a normal excretion level. In one patient 200 mg. daily was insufficient. It was also found that there was a greater decrease in the sedimentation rate of the red blood cells in treated patients in comparison with controls, but no noticeable clinical improvement was noticed after treatment with vitamin C [626]. Heise and Martin observed that large daily injections of vitamin C failed to protect guinea-pigs infected with virulent tubercle bacilli.

A vitamin C deficiency of from 1,800 to 3,600 mg. was observed in febrile tuberculous patients by Trautwein [627], who gave doses of 150 mg. to 300 mg. of vitamin C to correct the deficiency. After such treatment he recorded a decided improvement as shown by a gain in weight, fall in temperature, improved sedimentation rate and negative sputum. Similar observations were recorded by Pilz [628], Albrecht [629], Griebel [630], Bauer and Vorwerk [631]. Warns [632] treated twenty-six patients with bone and joint tuberculosis over a period of four and a half months with 250 mg. of vitamin C a day. No specific action on the tuberculous process was observed, although the general condition of the patients was improved.

Bogen and his co-workers [840] examined the effect of vitamin C on tuberculous lesions involving mucous membranes in nearly two hundred tuberculous patients. No marked effect on the course of the pulmonary disease occurred as a result of dietary supplements containing vitamin C, but ulcerated lesions of the mucous membranes in the tracheobronchi, larynx, intestine and rectum were stated to improve visibly in ninety per cent. of the patients. A group of patients served as a control to eliminate the psychological effect that might result from apparent improvement which often occurs when a new form of treatment is introduced. It was also observed that vitamin C deficiency appears to increase the incidence of certain serious complications of tuberculosis, and its abundant administration is therefore recommended by Bogen and his co-workers for their prevention or treatment. They are careful to point out, however, that vitamin C is by no means a cure for tuberculosis.

A very extensive study on vitamin C and tuberculosis was made by Sweany [635] and his colleagues, who examined two hundred and eighty-two tuberculous patients. They concluded that the increased requirements of vitamin C in the tuberculous patient are of the order of 150 to

200 mg. a day. In patients critically ill the vitamin C reserves were only 300 mg., compared with 5,000 to 6,000 mg. in a healthy person. It was thought that patients with advanced disease showed some benefit from being saturated with vitamin C, as shown by prolongation of life in the treated patients compared with the controls, as well as slightly more favourable clinical and laboratory observations. It is admitted, however, that little change could be observed radiologically.

Bakhsh and Rabbani [180] determined the vitamin C content of the urine of twenty-four tuberculous patients. Nineteen showed a deficiency. The administration of 150 to 200 mg. of vitamin C a day resulted in a gain in weight of ten of them, but it had no effect on hæmorrhage, and no appreciable changes could be detected radiologically or clinically.

McConkey [1012] has produced figures to show that cod liver oil and tomato juice are valuable remedies for preventing laryngeal and intestinal tuberculosis as a complication of pulmonary tuberculosis. To what extent vitamins A and C and the fatty acids in the oil play a part in this is unknown.

Erwin [636] and his colleagues at the Liverpool Sanatorium, encouraged by the reports of Continental writers, administered vitamin C to twenty-four selected tuberculous cases; some were acute cases with marked pyrexia and unlikely to improve on any known form of treatment, and others were chronic cases making no progress or even retrogressing. It was concluded that vitamin C was of no value at all in the treatment of tuberculosis and its complications, including hæmoptysis, and that saturation with the vitamin neither contributes to recovery nor retards retrogression. They concluded that the hypovitaminosis seen in tuberculosis is the result of pyrexia and toxæmia and is not specific.

Kaplan and Zonnis [850] also report that after correcting vitamin C deficiency in one hundred and one tuberculous patients over a period of six months, they were unable to observe any significantly favourable results, as judged by the usual clinical criteria, when compared with a group of control patients.

In conclusion it must be admitted that although most authors agree that there is an increased vitamin C requirement in tuberculosis, and that it is rational to satisfy it, the evidence for the therapeutic value of vitamin C in this condition is not convincing when viewed critically. Most of the work has not been controlled. Controls are essential in estimating the value of any therapy in a disease in which thirty per cent. of the patients recover spontaneously with fresh air, good food and rest. The average case of pulmonary tuberculosis undergoing sanatorium treatment is so apt to become apyrexial, gain in weight, and show subjective improvement that it is difficult to assess the value of a new form of treatment.

Whooping Cough. Vitamin C in a concentration of 80 mg. per litre inhibits the growth of *Hæmophilus pertussis* [649]; such a concentration cannot be reached in the blood, although it may be realized locally in the white blood cells according to Tonutti [108], who states that they may contain as much as 0.8 per cent. of vitamin C in active infections. Otani [650] states that the addition of vitamin C to the toxin of *Hæmophilus pertussis* diminished its virulence when injected into rabbits or guinea-

pigs. A hundred and nine cases of whooping cough were then given vitamin C as the only means of treatment. In mild cases from 50 to 100 mg. were given intravenously or intramuscularly. In cases of moderate severity from 100 to 150 mg. were administered, and in severe cases over 200 mg. were employed. These quantities were usually injected once a day, but sometimes two injections were given. Altogether from five to twelve injections were given, the period of treatment being between one and three weeks. In all eighty-nine cases (81·7 per cent.) were stated to have responded satisfactorily; forty (86·7 per cent.) reacted quickly; forty-nine (45 per cent.) not so quickly, although the vitamin C was considered to exert a definite effect; while in twenty (18·8 per cent.) no effect was observed. The results in uncomplicated cases were stated to be good, even when treatment was only begun a week after the spasmodic stage had developed. According to Otani when treatment was begun in the catarrhal stage, the spasmodic stage either did not develop at all, or else was of short duration. Among the cases which did not respond there were usually complications such as tuberculosis, measles, severe tonsillitis, etc.

Ormerod and Unkauf [651] of the University of Manitoba, state that vitamin C has a definite effect in shortening the paroxysmal period in whooping cough. In all twenty-seven cases were treated. Urinary excretion tests showed that most of the patients were excreting no vitamin C. After saturation with the vitamin the following clinical changes, which occurred in the order given, were recorded: marked reduction or complete arrest of vomiting; reduction or disappearance of night cough; reduction in number or intensity of whoops; reduction in number or intensity of day cough. An almost direct relationship was reported between the state of saturation with vitamin C and the intensity of the disease. In the opinion of these authors vitamin C therapy reduced the duration of the disease from a matter of weeks to days in most cases. The following scheme of dosage was used. As a routine 850 mg. were given by mouth on the first day; 250 mg. on the second and third days; 150 mg. on the sixth and seventh days; 125 mg. on the eighth and ninth days, and 100 mg. on the tenth day. The administration of 100 mg. daily was continued until complete recovery had occurred. Vermillion and Stafford [653] have used vitamin C in the treatment of twenty-six cases of whooping cough, and they report that the treatment seemed very effective in all but two of the patients, who apparently received little if any relief. The dosage employed was 150 mg. daily for the first three days; 120 mg. daily for the next three days; and 90 mg. daily until the symptoms subsided.

The only carefully controlled study on the effect of vitamin C fails to confirm these observations. Gairdner [654], at the Great Ormond Street Hospital for Children, treated twenty-one cases of whooping cough solely with vitamin C, twenty other cases serving as controls. The cases chosen had to fulfil one of the following conditions: *Hæmophilus pertussis* was recovered from a cough plate; a typical paroxysmal cough was noticed; there was a suggestive history together with a sublingual ulcer or a marked lymphocytosis. The value of the study was considerably

increased by noting the number of paroxysms. Each child was weighed weekly and this was considered a valuable guide to progress when vomiting and loss of appetite were often prominent symptoms. The dosage employed was 200 mg. daily during the first week, 150 mg. daily in the second week; and 100 mg. daily in the third and subsequent weeks. The average length of illness in the treated cases was thirty-five days compared with the forty-one in the control cases; the difference was not considered to be statistically significant. The average gain in weight of both treated children and controls was practically the same. Gairdner considered that there was no striking difference between the course of the disease in the cases treated with vitamin C and in the controls.

Respiratory Infections. Meyer [658] has demonstrated the presence of vitamin C in the tonsils and states that the vitamin C content can be increased by the oral administration of the vitamin. During infections the level falls rapidly. The results of recent investigations suggest that streptococci are less likely to be found in the tonsils when the vitamin C values of the blood are high and that when present in such cases they are seldom virulent [659]. Kramer [661] treated forty-two cases of tonsillitis exclusively by daily intramuscular injections of 500 mg. of vitamin C, which were given until the temperature fell. It dropped within three days in seventy per cent. of the cases. There were, however, no controls. Glazebrook and Thomson [816] also report that the duration of illness from tonsillitis was shorter in a group of patients who had received supplements of vitamin C, than in a control group that had not.

Certain authors have reported good results in the treatment of the common cold with large doses of vitamin C, *e.g.*, 800 to 1,000 mg. daily [662, 663]. Continental writers also state that the administration of large doses of vitamin C with quinine clears up attacks of influenza [668]. A mass experiment was made in Germany in 1941 and 1942 covering 1,570,000 mothers and infants and 2,180,000 children, who all received a hundred daily doses of 50 mg. of vitamin C prophylactically in the spring months [741]. In almost all areas a decrease in respiratory infections and skin eruptions in infants was stated to occur and epidemic diseases were considered to take a milder course. Pregnant and nursing women were stated to be subjectively benefited and lactation was thought to be improved. School children were said to be brighter, more energetic, and to have better appetites. In the Berlin area the blood vitamin C did not rise on the average until after two months' administration of the vitamin C, whereas in Bavaria, where the children are better nourished, the rise began almost at once. Unfortunately the experiment was a mass one and not controlled, so that results were based mainly on the subjective judgments of the general population.

It is possible that if the vitamin C reserves are very low and near the scorbutic level supplements of the vitamin may help to increase resistance to disease, although this was not the experience of Crandon [68], who noticed no greater incidence of respiratory infections on a diet very low in vitamin C than normally. There is, however, no clear-cut evidence that vitamin C or any other vitamin has any effect in the prevention or treatment of minor respiratory infections in adequately nourished persons.

Indiscriminate dosing with vitamin pills, which has been carried out in large factories and institutions for this purpose, is a waste of time and money. The carefully controlled studies of Glazebrook and Thomson [816] show that supplements of vitamin C have no effect in diminishing the incidence or duration of the common cold. Cowan, Diehl and Baker [742] gave two hundred students doses of 100 to 200 mg. of vitamin C daily and a control group had dummy tablets. In both groups there was a marked reduction in the number of colds experienced compared with former years. The difference between the two groups was not great and was possibly significant in favour of large doses of vitamin C. In the following year a group of one hundred and twenty students were treated daily with capsules containing 20,000 I.U. of vitamin A, 1.2 mg. vitamin B₁, 50 mg. of vitamin C, 2,000 I.U. of vitamin D and 200 micrograms of riboflavin. Another group acted as controls. There was no difference in the number of colds reported in the two groups.

The most valuable piece of controlled work on this subject has recently been published by Scandinavian workers. In 1948 Bergquist [1087] treated eight hundred and fifty-five males aged twenty to sixty for three months with vitamin C tablets and quinine, and the results, which were examined statistically, showed that neither with nor without quinine has vitamin C any prophylactic value in catarrhal complaints such as colds. Another worker [1088] reported that prophylactic vitamin C and quinine had a statistically significant effect in reducing the incidence of the common cold among factory workers, although the number of working days lost in the group receiving medication was no less than in controls.

Hammar and Schröderheim [1089] gave five hundred school children sufficient rose hip syrup to provide 50 mg. of vitamin C daily, and five hundred apple juice as a control. The test lasted from February to June, but there was no difference in the two groups in the frequency, intensity or duration of the common cold. The results were analysed statistically.

Dahlberg, Engel and Rydin [1090] carried out a mass experiment on two thousand five hundred Army conscripts, one-half of them receiving 200 mg. of vitamin C for twenty-four days, and then 50 mg. daily for three months, the other half receiving dummy tablets containing citric acid. No difference was found in the frequency or duration of colds, fever, endurance tests, or in diseases of any description. The work was carefully controlled and statistically evaluated. The control group suffered from considerable vitamin C deficiency as judged by urine acid saturation tests, yet their health was as good as those receiving the extra vitamin C.

Poliomyelitis. Jungeblutt [655] has shown that *in vitro* the R M V strain of poliomyelitis virus is inactivated by vitamin C. In *in vivo* experiments with rhesus monkeys vitamin C did have some protective effect if minimal infective doses of the virus were given; all the control animals developed some degree of paralysis, whereas sixteen per cent. of the treated animals showed no paralysis and were afebrile. The cords of the paralysed animals which had received vitamin C showed fewer pathological changes than those of the untreated ones. Sabin [656], however, found that both natural and synthetic preparations of vitamin C had no effect on the course of experimental poliomyelitis induced by the

nasal instillation of the virus in forty-six monkeys. Those whose store of vitamin C was depleted reacted in the same way as those receiving an adequate diet.

Heaslip [652] concluded from saturation tests on a number of subjects during a poliomyelitis epidemic in Australia that the vitamin C stores were lower than in normal controls. There was no evidence that administration of the vitamin had any effect on the clinical course of the disease.

Rheumatism. The vitamin C excretion of patients with active and convalescent rheumatism and rheumatoid arthritis has been found to be much lower than that of control subjects (p. 522).

Harris [485] and his colleagues have studied a total of a hundred and ninety-five cases of juvenile rheumatism (rheumatic fever, rheumatic heart disease, chorea and allied conditions). Ninety-two per cent. of the active cases showed a vitamin C excretion lower than normal; the figure for the convalescent cases was eighty-three per cent.

It is well known that adults and children of the upper, and therefore better fed, classes are not so subject to rheumatic infection as those of the lower classes, and the suggestion has been made that resistance to rheumatism may be in part a matter of nutrition. In 1934 Rinehart [638] and his colleagues found that guinea-pigs chronically infected with *β -streptococci* developed an arthropathy characterized by pain and swelling of the peri-articular tissues; in the presence of adequate vitamin C rheumatic lesions did not occur. The view was therefore advanced that rheumatic fever might be the result of the combined action of vitamin C deficiency and infection, particularly as in acute rheumatic fever the level of vitamin C in the blood was found to be considerably below normal [639]. Some of the experimental work of Rinehart and his co-workers has been confirmed by others, who do not agree, however, with his conclusions [640-642].

It has been pointed out by Schultz [640] that the lesions seen by Rinehart in his scorbutic guinea-pigs infected with streptococci are not identical with those of rheumatic fever, but merely resemble them. He suggests that it is more reasonable to regard the vitamin C deficiency as a result of rheumatic infection rather than as its cause, and he states that the administration of large amounts of vitamin C had no favourable effect on shortening the course or preventing attacks of rheumatic fever [643]. The effects of large doses of vitamin C prophylactically and therapeutically in rheumatic infections have been disappointing. Abt and his co-workers [152] gave forty-three patients with acute rheumatic fever and heart disease 300 to 600 mg. of vitamin C daily, but the clinical course of the disease was uninfluenced.

Perry [644] and Kaiser [645] have also concluded that although vitamin C deficiency may be observed in patients with rheumatic fever, it is not a significant ætiological factor. Keith and Hickmans [646] report that children with acutely active rheumatic fever excrete more vitamin C than convalescent patients, who excreted an amount approximately the same as controls in a similar age group. This increased excretion was probably due to the use of salicylates (p. 508).

Following the observations of Wolbach and Howe (p. 462) it has been suggested that a diminished vitamin C content of the blood may aggravate

the symptoms of arthritis by increasing the interstitial fluid, due to increased capillary permeability. Since the tensile strength of the finer vascular bed is largely due to its connective tissue support, lack of intercellular material may result in loss of capillary tonus and increased capillary permeability. Rinehart believes that a chronic vitamin C deficiency is also an important ætiological factor in rheumatoid arthritis. Low blood levels have been observed in many cases of rheumatoid arthritis [436]. Glazebrook and Thomson [816] have shown that the incidence of rheumatic fever was lower in a large group of adolescents receiving supplements of vitamin C than in a control group that did not. While most observers have failed to observe any clinical response after administering vitamin C, Rinehart [436] states that in rheumatoid spondylitis vitamin C has a favourable effect on the sedimentation rate, capillary strength, weight, general condition and arthritis. Hall [436] and his colleagues saturated arthritics for eight months with vitamin C, but no clinical improvement was observed that could be attributed to the vitamin. Some patients improved during this period but others continued unchanged or became worse, as shown by the condition of their joints, failure to gain weight and increased sedimentation rate. On the other hand Sherwood [647], who studied the effect of vitamin C on fifty arthritics, found that improvement occurred in some and was always preceded or accompanied by a return of the blood vitamin C to normal. Hare and Williams [648] gave high vitamin C diets to six patients with rheumatoid arthritis and reported a definite clinical improvement in five of them. Unless a large series of cases is taken and observations carefully controlled, it is difficult to assess the value of any treatment in arthritis, with its well-known periods of exacerbation and remission.

Herpes. Dainow [461], of the Dermatological Clinic in Geneva University, has treated cases of herpes febrilis with daily doses of 100 mg. of vitamin C intravenously, and he states that the condition cleared up in two to three days. He also gave the same dose in cases of herpes zoster and states that after three injections the vesicles dried up and the pain rapidly disappeared. These observations have not been confirmed.

Malaria. It has been shown that the vitamin C requirements are increased in malaria [462, 463], particularly during rigors. Mohr and Kühner [463] observed that the administration of vitamin C caused a diuresis, reticulocytosis, and an increase in the amount of hæmoglobin in malaria patients. They suggest that 100 mg. of vitamin C a day should be administered. A similar improvement in the blood picture was noted by Gerdjikoff [462]. It has not been satisfactorily shown that the administration of vitamin C has any effect on the course of the disease.

Typhoid Fever. Najib-Farah [666] observed the frequent occurrence of symptoms resembling those of adrenal insufficiency occurring during the course of typhoid fever. He therefore used adrenal cortex extract and vitamin C in large doses in the treatment of fifteen cases of typhoid. Treatment, which was started immediately the diagnosis was made, consisted of 5 to 10 c.c. of adrenal cortex extract intravenously and 500–1,000 mg. of vitamin C given daily for five to twelve days. The results

were claimed to be excellent. Szirmai [667] gave typhoid patients 300 mg. of vitamin C a day intravenously for four to five days and states that intestinal hæmorrhages were almost completely prevented. Other workers have failed to prevent gastro-intestinal hæmorrhages with vitamin C.

Dental and Oral Conditions. The importance of vitamin C in the development of the teeth is discussed on p. 462.

Although there is general agreement that an adequate intake of vitamin C is necessary for normal tooth structure and growth there is no clear cut evidence that vitamin C deficiency plays any part in the ætiology of dental caries in human beings. Hanke [671], Korn [55] and Bucher [56] believe that a liberal intake of vitamin C is of value in the prophylaxis of dental caries. Korn and Bucher are of the opinion that the increase of dental caries in pregnancy is due to vitamin C deficiency. They examined a number of pregnant women and noted a high incidence of dental caries in those showing a vitamin C deficiency, as shown by saturation tests. Bucher states that he observed a diminished incidence of dental caries in pregnant women receiving supplements of vitamin C every day. None of these studies, however, was controlled. Schiötz [674] observed that the teeth of children fed on liberal diets containing plenty of milk, orange juice, apple and carrot were less carious than those of children from poor districts whose food lacked these articles of diet. There are so many dietary factors involved here that the beneficial effects of the diet cannot be ascribed solely to vitamin C. Controlled animal studies by Boyle [49] show that on low intakes of vitamin C the deposition of dentine in the incisor teeth is much retarded, but the enamel continues to be deposited at the normal rate. No correlation was found between vitamin C deficiency and the incidence of dental caries. According to Sandberg and Dagulf [51] no correlation exists between the values for blood vitamin C and either the absolute frequency of dental caries or the increase of dental caries studied over a period of two years.

It has been recognized from early times that the gums are affected in scurvy. While the existence of gingivitis in scorbutic or sub-scorbutic human beings and guinea-pigs is not doubted, there is considerable difference of opinion on the part played by minor degrees of vitamin C deficiency in the ætiology of gingivitis as met with in general medical and dental practice. A careful clinical examination should be made before attributing bleeding gums to vitamin C deficiency. A mistaken diagnosis of vitamin C deficiency was made in four cases of this kind; the true diagnosis was uræmic toxæmia [1065].

Earlier workers recorded that supplements of fruit juices or synthetic vitamin C in doses of 100 to 400 mg. daily had a favourable effect on gingivitis and the healing of bleeding and sore gums [53, 671-677, 807]. Vitamin C deficiency, as evidenced by a diminished excretion or low blood levels, was also recorded by a number of observers in patients with these conditions [58, 678, 675, 805, 812]. Much of this work was uncontrolled, and since in some cases local treatment was also carried out at the same time, it is difficult to evaluate. A number of workers have failed to find any significant relationship between the intake of vitamin C and the incidence of gingivitis [68, 680, 748, 779, 1016]. Burrill [1017], from

observations on nearly 1,400 patients, noted that vitamin C plasma levels in patients with gingivitis and periodontal disease tend to be lower than controls. The differences between the two groups were, however, small and probably not statistically significant. The seasonal variations in the plasma vitamin C and the incidence of gingivitis were such that no causal relationship between a low level of vitamin C nutrition and gingivitis could be established. Burrill states that a patient likely to neglect his mouth will also neglect his diet; vitamin C subnutrition and oral infection originate from the same cause rather than from one another.

Roff and Glazebrook [57, 678] examined six hundred boys on the training ship *Caledonia* and observed that stomato-gingivitis associated with vitamin C deficiency was common amongst these boys, whose normal vitamin C intake averaged only 25 mg. a day. Using the test dose method of Harris (p. 545) the average deficiency was approximately 4,000 mg. per boy. Three hundred of the boys were saturated with vitamin C (200 to 300 mg. a day), and then the dose reduced to a maintenance level of 50 mg. a day, so that the total vitamin C intake was 75 mg. a day after saturation. The same number of boys in a control group received the same diet, but no supplements of vitamin C. Before treatment the incidence of gingivitis and gingivo-stomatitis in the group was 17.6 per cent.; after six weeks' treatment with vitamin C it fell to 4.9 per cent. The corresponding figures in the control group before and after receiving dental treatment but no supplements of vitamin C were 16.8 and 12.64 per cent. The boys in this group suffering from gingivo-stomatitis were then treated with vitamin C, and usually within fourteen days a characteristic colour change was observed in the gums, which lost their deep red colour, became firmer and assumed their normal pink appearance. Roff and Glazebrook are of the opinion that marginal gingivitis is due to bad dental hygiene, since it usually responds to dental treatment, whereas gingivo-stomatitis does not. The latter is probably a sub-scorbutic manifestation.

These observations were supported by Campbell and Cook [679], who distinguished between simple gingivitis and ulcerative, ulcero-membranous or fuso-spirochætal gingivitis (Vincent's disease). According to Campbell and Cook, in simple gingivitis the gums are sore and bleed on pressure and the condition clears rapidly with doses of 800 mg. of vitamin C daily. With the ulcerative type pain and bleeding are more intense, there is a characteristic foetor and smears show pus, spirochaetes and fusiform bacilli. Treatment is more difficult and does not yield to vitamin C alone, but requires in addition local treatment with chromium trioxide and hydrogen peroxide or magnesium sulphate paste.

Stuhl [892] and Buchanan [1018] record a high incidence of vitamin C deficiency in patients with ulcerative gingivitis in the British Army and Navy. This high incidence of vitamin C deficiency is general, owing to the deplorable condition of catering and cookery in the Services, e.g., army stew, which is often cooked for two to three hours. The vitamin C deficiency is probably not directly related to gingivitis. Stuhl and Buchanan state that the effects of local treatment (gentian violet, two per cent.) are enhanced by the administration of vitamin C. Buchanan states that ulcerative gingivitis does not clear up with normal dental treatment

and is progressive unless local treatment is supplemented by treatment with 700 mg. of vitamin C daily until the patient is saturated.

There is considerable difference of opinion on the aetiology of ulcerative gingivitis (ulcerative gingivo-stomatitis, Vincent's disease, "trench mouth," fuso-spirochætal gingivitis). Pincus [1019] doubts whether it is associated with vitamin C deficiency, since the condition may clear up with local treatment alone and the use of vitamin C without the latter gives variable results. Pincus observed two hundred men with ulcerative gingivitis and states that many were getting enough vitamin C. Cuthbert and Williams [1020] also failed to show any degree of vitamin C sub-nutrition in sailors suffering from ulcerative gingivitis. The vitamin C nutrition was no worse in these men than in non-infected healthy seamen. They found that the administration of vitamin C did not accelerate cure of the gingivitis, and they were inclined to think that the chief factor in the spread of the disease is contagion, *e.g.*, due to "French kissing," inadequate washing of crockery and dirty oral habits. It is possible that vitamin C deficiency results in lowered resistance of the gums to infection, but that once the infection has occurred large doses of vitamin C are ineffective in curing the condition, just as they do not affect the clinical course of the infectious fevers (p. 556). The "official" view is that "there is ample evidence that ascorbic acid therapy is ineffective" in the treatment of ulcerative gingivitis [1021].

Kent [1022] believes that much confusion over treatment has resulted because there are two clinical entities, latent scorbutic gingival ulceration and ulceromembranous gingivitis or Vincent's disease. In the former local therapy fails and the response to vitamin C is dramatic; in the latter local therapy is necessary. Kent describes the characteristic picture of latent scorbutic gingival ulceration—bleeding, injected, swollen, atonic gums; spongy, pale and "frosted" gingival margins and intergingival areas; marginal gingival œdema; ulcerated patches; lack of cervical adenitis; negative smear; other clinical or laboratory signs of vitamin C deficiency. In Vincent's disease the gums are covered with irregular ulcers covered by a yellowish grey slough which forms a pseudomembrane, removal of which leaves a raw surface which bleeds briskly. Ulcers appear in mucous folds, crevices, periodontal pockets and cracks. The gingival margins are the common sites of ulcers. Ulceration may spread to the cheeks, lips, tongue or palate. A section of a typical ulcer shows necrotic debris, spirochætes and fusiform bacilli. Kent found that topical therapy and vitamin C gave variable results in this form of gingivitis. Sometimes it cured mild and moderate cases. He believes that the best results are obtained with mapharsen and vitamin C in doses of 300 to 600 mg. daily.

MacDonald [1023] noted that there was a high incidence of bleeding gums and gingivitis in naval ratings. He was unable to show that it was related to vitamin C deficiency and the condition cleared up with local treatment. Ungley and Horton [1016] made similar observations on naval patients with sore and bleeding gums, in whom scurvy or "subscurvy" was absent. Local causes, such as infection and calculus, were sufficient to account for the gum condition, and both vitamin C and nicotinic acid were ineffective. Day and Shourie [1024] found that vitamin C was

ineffective in the treatment of gingivitis and associated conditions in Indian children. Stammers [1025] strongly advocates local treatment in ulcerative gingivitis. He finds vitamin C and nicotinic acid therapy disappointing, with a response rate of only eighteen per cent. Recently Stamm, Macrae and Yudkin [1042] found that twenty per cent. of a group of nearly three thousand R.A.F. personnel suffered from bleeding gums, but no greater improvement was obtained by treatment with vitamin C than with inert control tablets. Those with "sponginess" as well as bleeding of the gums showed no more improvement after taking vitamin C than when given control tablets. These workers concluded that the incidence of bleeding gums is not related to vitamin C deficiency.

These conflicting views on vitamin C and gingivitis are undoubtedly due to confusion over the aetiology and symptomatology of inflammatory conditions of the gums and the absence of established standards for the normal gum. Many investigators do not state the exact type of lesion treated and the criteria for assessing recovery, or for assessing vitamin deficiency. It is quite likely, as Kent points out, that there are two clinical entities, which have been confused—latent scorbutic gingival ulceration, which responds to vitamin C therapy, and true Vincent's disease, which is primarily infective and is not directly associated with vitamin C deficiency, although in some cases vitamin C therapy may help in conjunction with local therapy.

The recent observations of Farmer and his colleagues [1078] suggest that there is probably no connection at all between vitamin C deficiency and dental lesions, excluding of course frank scurvy. They kept volunteers on a scorbutic diet for five months but failed to observe any changes in the gums, teeth or bone of the jaw as observed with the naked eye, radiologically or with the biomicroscope. The plasma vitamin C and the vitamin C in the white cell layer were zero. These observations suggest that the oral conditions ascribed to vitamin C deficiency by some writers are in reality due to pre-existing caries and gingivitis or improper dental hygiene and are not related to vitamin C deficiency.

It is stated by Campbell and Cook [853] that the oral administration of vitamin C to patients before and after dental extractions plays an important rôle in the healing of the wounded gum tissue and the absorption of the alveolar bone margins. They also report that pain and bleeding, particularly persistent hæmorrhage after extractions, are considerably reduced. The patients were given 300 mg. of vitamin C daily until "saturated" (p. 544) and then 100 mg. daily as a maintenance dose. Persistent hæmorrhage was treated with 500 mg. of the vitamin.

The use of nicotinic acid in oral conditions is described on p. 428.

Hæmatology. In scurvy erythropoiesis is depressed in the bone marrow; the anæmia usually responds to treatment with vitamin C (p. 526). Recent work in this country has drawn attention to the increased incidence of anæmia during the war, particularly among pregnant women. An inadequate intake of iron has been suggested as the cause, but it may be that vitamin C deficiency plays a part as well [999]. Israels [999] suggests that any patient whose anæmia does not respond to iron should be given vitamin C. Dyke and Della Vida [784] state that a number of

pernicious anæmia patients, in spite of their maintenance dosage of liver, showed a progressive fall in the red blood count. This was checked by giving 100 mg. of vitamin C daily for a month, the red blood count increasing by 1,000,000 per c.mm. These observations suggest that in pernicious anæmia, even with ample dosage of liver extract, hæmopoiesis is subnormal if there is any degree of vitamin C deficiency.

The prompt response of the petechial hæmorrhages of scurvy to the administration of vitamin C suggested its use in hæmorrhagic conditions such as Schönlein's and Henoch's purpuras, hæmophilia and idiopathic thrombocytopenic purpura. It was argued that vitamin C reduced the capillary permeability and increased the platelet formation in the bone marrow. The earlier reports on the value of vitamin C in purpura and hæmophilia only mention a few cases and do not bear statistical examination. Böger and Schröder [685] report a case of thrombopenic purpura that improved after four daily injections of 150 mg. of vitamin C; after daily injections of this dose for a month the thrombocyte count rose to 804,000 per c.mm. Doses of 1,000 to 1,500 mg. a day were used by Winckelmann [686], who considers that the failures of others were due to too low dosage. Vogt [687] treated two cases of thrombopenic purpura with 100 to 800 mg. of vitamin C daily with apparently favourable results; the diagnosis of one case, however, was doubtful. Four cases of idiopathic thrombocytopenic purpura were treated by Miller and Rhoads [688], who reported clinical improvement in two of the cases and an increase in the thrombocyte count. An increase in the thrombocyte count and decreased bleeding in cases of thrombocytopenic purpura treated with 200 mg. of vitamin C a day were observed by Böger and Morton [689]. Good results are also reported by Glanzmann [690], Guggisberg [691], Falconer [692], Engelkes [693], and Schuchard [694]. It must be pointed out that the possibility of spontaneous recovery makes evaluation of the results difficult. The early enthusiastic claims have not been substantiated by later workers. Davidson [695], Vaughan [696], Wright and Lilienfeld [697], Stephens and Hawley [698], Rosenthal [699], Abt and Farmer [700], and Walther [701] state that practically no improvement occurred in cases of purpura treated with vitamin C. Rosenthal [699] treated ten cases of chronic purpura and Scarborough [1026] fifteen with vitamin C without success. Wright and Lilienfeld [697] describe a patient with thrombocytopenic purpura who received 100 mg. of vitamin C daily for thirty-eight days without any improvement. Finkle [702] found no evidence of vitamin C deficiency in chronic purpura.

Claims have been made that hæmophilia responds to treatment with vitamin C, but the accounts are usually those of success in isolated cases [685, 703, 704]. Witts [719] and Armentano [718] have found vitamin C ineffective in hæmorrhagic states not associated with vitamin C deficiency.

Vitamin C has been used in the treatment of other hæmorrhagic conditions. Vogt [687], Müller [705], Hükel [706] and Neumann [707] describe its use in gynæcological hæmorrhage (pre-climacteric and climacteric menorrhagia, hæmorrhage from myomata) and epistaxis. Deeny [708] has carried out an experiment on thirty-two women of the industrial class, all of whom were suffering from menorrhagia and showed a vitamin C

deficiency. After saturation with vitamin C it was found that twenty of the thirty-two cases were restored to a clinically normal condition, eight were improved and four had no change. In those patients improved there was a diminution in the duration of the menstrual periods, an increase in the percentage hæmoglobin, and a disappearance of the pain and symptoms associated with dysmenorrhœa. Deeny [708] believes that vitamin C deficiency may cause menorrhagia, either by impairing the health of the endometrial blood vessels or by effect on the endocrine system (progesterone). His figures have been examined and shown to be statistically significant.

In the opinion of some workers essential hæmaturia is a manifestation of vitamin C deficiency, and yields to treatment with this vitamin [698, 709-711]. It is claimed that deficiency causes an abnormal permeability of the kidney glomeruli. Burkland [709] states that he successfully treated four cases of essential hæmaturia, which had been present for four weeks and was without obvious cause, with intravenous injections of 100 to 300 mg. of vitamin C a day. Burkland considers that essential hæmaturia might be considered as a manifestation of subclinical vitamin C deficiency. Lane [710] also describes patients with vitamin C deficiency suffering from essential hæmaturia. On cystoscopic examination their bladders showed patchy purpuric rashes that oozed blood, but after ten days' treatment with vitamin C the urine was free from red cells and the bladders were normal in appearance. Miller and his colleagues [712] were unable to observe any improvement in the hæmaturia of nephritis after administering vitamin C in doses of 1,000 mg. orally or 100 mg. intravenously for ten days.

It has been stated that vitamin C is effective in the treatment of pulmonary and gastro-intestinal hæmorrhage [718, 715, 716], except in very severe cases. These conditions cease spontaneously, or else the patient succumbs, and as the majority of patients do recover it is difficult to evaluate the effect of vitamin C tried in small groups of cases. Anderson [717] found vitamin C of no value in the treatment of gastro-intestinal hæmorrhage.

Deeny and his collaborators [1028] have described two cases of familial "idiopathic methæmoglobinæmia, a very rare disease, one of the "inborn errors of metabolism," characterized by a permanent slaty-blue colour of the skin, and the presence of methæmoglobin in the blood. The general health in this disease does not suffer as the total blood pigment is usually increased so that a normal amount of functioning oxyhæmoglobin is available. Oral administration of sodium bicarbonate and 300 to 400 mg. of vitamin C daily resulted in disappearance of the bluish colour of the skin, which became normal in a few weeks and remained so for a year while under treatment. In one case the methæmoglobin was reduced from forty-three per cent. to six per cent. of the total blood pigments in a month. These were the first cases of familial idiopathic methæmoglobinæmia to be described in this country. Another case, responding to treatment with 300 to 600 mg. of vitamin C daily, has been described by King, Gilchrist and White [1029].

and Gaehtgens [720] describe a case of myeloid leukaemia

in a woman of thirty-five, whom they treated with 100 mg. doses of vitamin C twice daily for ten days. The leucocyte count dropped on the fourth day to one-half the value on admission and after eight days reached normal. Similar results are reported by Thiele [722]. A striking reduction in the leucocyte count in patients with pathologically increased leucocyte counts (leukæmia, pneumonia) was observed by Schnetz [107] after administering vitamin C. Vogt [721] and Kalk [724] state that vitamin C has no effect in myeloid and lymphatic leukæmia apart from improving the general condition of the patient.

Cases of leucopenia have been stated to respond to treatment with vitamin C. According to Schnetz [107] vitamin C keeps the leucocyte count at normal levels, since it reduces the count in leukæmia, and raises it in leucopenia. Vogt [721] and Carrie [728] state that leucopenia resulting from X-ray treatment can be corrected by administering vitamin C.

Kalk [724] gives full reports on six cases of granulocytopenia treated with intravenous injections of 500 to 1,000 mg. of vitamin C daily, with good results, including an increase in the total leucocyte and granulocyte counts. In one case a relapse occurred when treatment was stopped, but the patient recovered again when treatment with vitamin C was resumed. According to Schröder [111] and Rietschel [112] intravenous injections of vitamin C in doses of 400 mg. to 700 mg. produce a thrombocytosis.

The possibility that vitamin C may play a part in maintaining the red blood count at normal levels and its use in the treatment of polycythæmia have been discussed on p. 476.

Vitamin C in Dermatology. During his experiments on induced scurvy Crandon [68] noted that after a hundred and thirty-four days on a diet practically free from vitamin C small hyperkeratotic papules began to develop over the buttocks and posterior aspects of the calves. Noticeable fragmentation of the hairs and marked dryness of the skin were also noted. It was proved that these skin lesions were not the result of vitamin A deficiency, as 80,000 units of this vitamin were being taken daily. The administration of vitamin C rapidly cured these skin lesions.

It has been suggested that vitamin C is intimately connected with pigment formation and pigmentary disorders of the skin (p. 492), in which it is encountered for the most part in the basal layer of the epidermis and in hyperpigmented structures. The formation of pigment in the skin depends on the ability of the melanoblasts to produce it, probably through the oxidation of certain organic substances, including *l*-8 : 4-dihydroxy-phenylalanine ("dopa"). All pigment producing cells stain black with *dopa* reagent. According to Szent-Györgyi [281] vitamin C inhibits the formation of pigments formed through the oxidation of phenolic substances, such as *dopa*, and by virtue of its location in the pigment areas of the skin might conceivably be able to prevent oxidation and subsequent pigment formation. This has been verified by *in vitro* experiments [282-284]. On the other hand the hyperpigmentation of the nipples and areolæ of castrated guinea-pigs receiving oestrogens was uninfluenced by giving vitamin C [729]. Cornbleet [780] found that human skin contained less vitamin C after ultraviolet irradiation. He further observed that a section of skin from a negro injected with vitamin C reacted more to silver nitrate than a

section of skin from a white person who had received a similar amount of vitamin C. He inferred from this that the pigment fixed the vitamin C in the skin.

If vitamin C has an inhibiting effect on pigment production it might explain the hyperpigmentation that occurs in scurvy and Addison's disease. Low vitamin C levels have been reported in Addison's disease (p. 492), and it is stated that the pigmentation of this condition is diminished by the administration of vitamin C [285, 286].

Reiss [508] observed a depletion of vitamin C in patients suffering from psoriasis; clinical improvement was stated to follow administration of the vitamin. A favourable response to treatment with vitamin C in three cases of psoriasis is described by Lutz [781], who gave one patient a total of 42.6 grams over a period of ten weeks. The lesions, however, subsequently recurred. Volpe [732] and Mage [733] also report cases in which vitamin C produced clinical improvement, although Madden [785] found that it had no effect at all in his cases. These observations based on few cases require confirmation.

A deficiency of vitamin C has been suggested as a cause of urticaria by Rosenberg [786], who determined the blood vitamin C in seven patients with this condition and found what he considered to be low values. He reports good therapeutic results from the administration of vitamin C. As vitamin C has no effect on capillary permeability (p. 541) there is no reason to believe that it should be effective in the treatment of urticaria.

Wernick [787] determined the vitamin C excretion in patients with lupus vulgaris, and found that out of fourteen cases, it was low in twelve. More vitamin C than normal was necessary to produce a rise in the urinary excretion of vitamin C, but the clinical improvement resulting from the administration of the vitamin was not impressive. Similarly Finkle [788] found that the vitamin C excretion was very low in five cases of acute lupus erythematosus in comparison with that of controls suffering from other diseases, but administration of the vitamin had no favourable effect on the course of the disease.

Hagemann [789] gave doses of 200 mg. of vitamin C intravenously for three days in five cases of erythema multiforme, followed by 6 gr. of aspirin three times a day, and he claims that a rapid cure was effected. Good results were reported by Wagner [740] in the treatment of eczema in ten breast-fed infants by improving the state of nutrition of the mother by giving her vitamin C and administering 150 mg. of the vitamin a day to the infant. Other types of eczema were stated to respond to vitamin C therapy in doses up to 800 mg. a day.

Lever and Talbott [744] examined the blood vitamin C in a hundred and eighty-one patients suffering from various skin lesions, including dermatitis, psoriasis, urticaria, lupus vulgaris, lupus erythematosus, eczema, exfoliative dermatitis, pemphigus and acne vulgaris. The results were compared with those of sixty-eight apparently healthy persons. Great variations were found, but fifty-five per cent. of those with skin lesions had levels below 0.8 mg. per 100 c.c. of blood—a level considered by many to be scorbutic (p. 550); the average was only 0.86 mg. However, there was no significant correlation between the blood vitamin C and the

development of the skin lesions, and large amounts of the vitamin were given to eighteen patients showing low levels, but without any improvement in their clinical condition. Doses of 200 mg. a day were employed over periods of from two to ten weeks. Lever and Talbott reject the view that a deficiency of vitamin C is a contributory ætiological factor in the production of certain diseases of the skin. Braestrup and Hansen [745] noted that patients with generalized skin conditions had consistently low renal thresholds for vitamin C; they observed no beneficial effect on the clinical condition as a result of administering the vitamin.

There are reports that the erythrodermia and dermatitis resulting from treatment with arsphenamines and gold compounds respond to vitamin C therapy. The subject has already been discussed on p. 482 in connection with the detoxicating properties of vitamin C.

Ophthalmology. Vitamin C plays an important part in the nutrition of the ocular tissues according to Friedenwald, Buschke and Michel [962]. They state that the interstitial tissue of the stroma of the ciliary processes contains a group of reducing substances, one component of which is vitamin C, which act as a mediating system, facilitating the oxidation-reduction interaction between the stroma cells and the stroma-epithelium barrier. According to these workers vitamin C deficiency in the guinea-pig causes the disappearance of vitamin C from the ocular tissues long before any symptoms of scurvy appear. This loss of vitamin C from the secretory system of the eye results in a decrease in the rate of transfer of the basic dyes from the stroma to the epithelium and in a decrease in the rate of secretion of the intraocular fluid.

The healthy lens has been shown to be particularly rich in vitamin C, the amount of which is greatly reduced or entirely absent in the lens that has developed cataract [489, 746]. According to Bellows [489] patients with cataract also show lower vitamin C levels than do control patients in the same age group and economic status. He has also shown that the onset of the cataract that develops in rats fed on large quantities of galactose can be delayed by the administration of vitamin C. Other workers state that vitamin C has no direct bearing on the ætiology of cataract in animals [747]. It is possible that vitamin C is in some way essential for the metabolism of the lens, but there is no convincing clinical evidence that it arrests the progress of cataract in the human eye, although Josephson [750] believes that the administration of up to 800 mg. of vitamin C a day to patients with cataract causes marked improvement within a week. The same writer states that cataracts caused by poisoning with dinitrophenol respond to treatment with vitamin C. According to Friedenwald [749] the visual acuity of diabetics, in whom cataract is common, is improved by the administration of vitamin C, although Owens [257] and his co-workers were unable to confirm this.

In the course of routine refraction tests Bouton [491] observed clouding of the optic media associated with impaired vision in a number of patients, who in comparison with controls showed a definite vitamin C deficiency. The patients were given 800 to 850 mg. of vitamin C daily for four to eight weeks, and at the end of that time re-examination showed that in sixty per cent. the eyesight improved, as measured by reading tests,

ophthalmic examination, and subjective reactions. Patients with senile cataract were not improved by the treatment, all improvement being due according to Bouton to clearing of the optic media, and to the beneficial effect on the retinal vessels and the head of the optic nerve. The improvement, if it occurred at all, was stated to have set in within the first two weeks of treatment.

Eleven cases of retinitis pigmentosa were examined by Dax [752], who found a decreased excretion of vitamin C in all of them. The administration of vitamin C for a month did not bring the excretion up to normal, and it had no curative effect on the condition.

Yudkin [753] states that vitamin C is valuable in ocular diseases where the condition is associated with vascular disturbances, *e.g.*, hæmorrhagic retinitis and choroiditis, hæmorrhage into the vitreous from local arteriosclerosis and general hypertension, and œdema of the macular region produced by a vascular decompensation. These conditions were stated to improve with large doses of vitamin C (800 to 500 mg. daily). If the hæmorrhage is due to other causes vitamin C is not expected to have any effect. Traube [754] reports a case of severe conjunctival bleeding that responded to treatment with vitamin C. It has been shown that vitamin C is not associated with capillary resistance (p. 541), and it is therefore difficult to understand why it should be effective in the treatment of retinal and conjunctival hæmorrhage.

Vitamin C is known to exist in high concentrations in the human cornea as well as the lens, and on the assumption that it is an important factor in the nutrition of the structure of the eye Lyle and McLean [755] used it in cases of inflammatory conditions of the cornea. Among the conditions treated were dendritic corneal ulcer, disciform keratitis, sclerosing keratitis, superficial punctate keratitis, phlyctenular keratitis, post-vaccinal keratitis, and corneal ulceration, all of which occurred as complications to injuries of the eye in airmen. Doses of 500 mg. of vitamin C were given once a day intravenously, as well as ordinary local treatment, until active inflammation of the eye had ceased, after which the vitamin C was given orally. Lyle and McLean state that "improvement in most cases is almost dramatic." In most cases there was no reason to believe that a vitamin C deficiency existed, and the beneficial results were attributed to flooding the body with excess vitamin C. The treatment was found useless in iritis; in fact one case appeared to be aggravated. Local treatment was also carried out and undoubtedly contributed to the improvement of the patients.

Livingstone and Walker [756] state that direct mustard gas contamination of the eye can be considerably mitigated by high doses of vitamin C. No human trials have been possible so far and the only experiments have been on rabbits. Twenty minutes after exposure of the eyes of four rabbits to mustard gas an intravenous injection of 500 mg. of vitamin C was given; six daily injections of 500 mg. were subsequently administered. In comparison with controls which were treated by irrigation this treatment was stated to have a remarkable effect in preventing the spread of keratitis, the progress of lid inflammation, and secondary infection. Mann and Pullinger [757] repeated the experiments

of Livingstone and Walker, but could not confirm their claim that injections of vitamin C influence the progress of mustard gas lesions of the eye.

The improvement in dark adaptation said to be brought about by vitamin C in subjects with poor adaptation has been referred to previously (p. 497).

Metabolic Diseases. Reference has been previously made to the use of vitamin C in the treatment of Addison's disease and diabetes (pp. 492, 495). Since the metabolic rate is raised in hyperthyroidism and the vitamin C level is low, it would appear rational to increase the intake of the vitamin in this and all conditions associated with increased metabolism (p. 522).

A decreased excretion of vitamin C and presumably an increased requirement for the vitamin have been observed in patients suffering from malignant disease [440]. It has also been found that the amount of vitamin C in actively growing and metabolizing tissues and malignant tumours is greater than that of normal tissues or of benign tumours [289]. According to Ryo [758] vitamin C inhibits the growth of tumour cells injected into experimental animals. These observations would suggest that the vitamin C intake of patients with cancer be increased. Deucher and Schneider [290] observed a marked improvement in the general condition of patients with cancer after the administration of very large doses (1 to 4 grams) of vitamin C, but no effect was observed on the course of the disease.

Allergic Conditions. Evidence that anaphylactic shock in sensitized animals can be prevented or minimized by the injection of vitamin C is confusing. It has been stated that animals receiving small amounts of vitamin C are more sensitive to a second dose of horse serum than normal animals receiving adequate supplies of the vitamin [759]. It is stated that large doses of vitamin C prevent, or at any rate minimize, the anaphylactic shock that occurs in animals sensitized to horse serum; the greatest degree of protection is said to be provided when the vitamin is given at the same time as the sensitizing dose of serum [760, 761]. Hochwald [767] reports that vitamin C is effective if given intraperitoneally in doses of 100 mg. between fifteen and forty-five minutes before the shock dose. While more than ninety per cent. of the controls were lost after receiving the second dose of serum, about two-thirds of the animals treated with vitamin C lived and showed no signs of shock. Others have failed to confirm any association between vitamin C and anaphylaxis [762-764]. According to Yoshikawa [764] the continued use of large doses of vitamin C may inhibit anaphylactic shock, while smaller doses intensify it.

Friedmann [828] has shown that in guinea-pigs a deficiency of vitamin C causes a definite loss of smooth muscle responsiveness to non-specific stimulation and Raffel and Madison [829] have demonstrated that vitamin C influences the antibody response to specific antigenic stimulation. Vitamin C deficiency therefore directly influences two of the basic factors concerned in anaphylaxis, namely, smooth muscle contractility, and antibody production.

These animal experiments suggested the clinical use of vitamin C in allergic conditions. Schiødt and Søgaard [766] studied the effect of vitamin C on pneumonia patients who had received anti-pneumococcal

serum. While seven control patients all had serum sickness, with well-marked exanthema and arthritic symptoms, three out of nine patients treated with vitamin C did not, and the remaining six only developed it slightly. The vitamin C was given intravenously in doses of 100 to 200 mg. after the temperature had fallen for from five to eighteen days. Szirmai [765], on the other hand, failed to prevent serum sickness with vitamin C.

Several reports have appeared on the use of vitamin C in the treatment of bronchial asthma, but until recently no studies have been made on the state of vitamin C nutrition before and during treatment. The rationale of its use is described by Hochwald [767]. Asthma is an example of a hyperergic manifestation, which he describes as a reaction produced in a sensitized organism, characterized histologically by fibrinous inflammation and biochemically by an increase in the fibrinogen and globulin of the blood. Hochwald observed that the administration of vitamin C to animals in which a hyperergic state had been produced by the injection of foreign proteins caused a lowering of the serum fibrinogen and globulin. This suggested its use in the treatment of asthma. Hochwald states that vitamin C is of value in preventing symptoms if administered regularly, and if given intravenously in doses of 500 to 1,500 mg. is useful in aborting symptoms. Epstein [768], using large doses of vitamin C, was unable to confirm these results; according to him it was of no use alone in the prevention or cure of asthmatic attacks, but he considered that it was a valuable adjuvant to other forms of treatment in doses of 100 mg.

Hagiesco [769] and his associates used vitamin C for the treatment of twenty refractory cases of asthma, and they report that treatment was successful in fifteen of the cases, uncertain in two, and valueless in three. To stop an attack of asthma they give an initial intravenous injection of 200 mg. to 300 mg. of vitamin C followed by a second injection of 100 mg. to 200 mg. a quarter of an hour later. In cases that react well to treatment with vitamin C the attacks cease entirely after from one to five injections, and they become less severe and less prolonged. It is also recommended that injections of vitamin C be given to patients when they have prodromal symptoms. The suggested dosage in between attacks is 100 to 200 mg. intravenously. At least five to six injections a month are recommended. Several other publications describing the successful use of vitamin C in asthma have appeared [770]. It is possible that vitamin C has a spasmolytic effect in asthma, since it prevents bronchospasm in guinea-pigs injected with acetylcholine, which normally constricts the bronchi [771]. Kennedy [848] claims that a suspension of vitamin C and adrenaline is effective in certain types of asthma and has a more prolonged action than adrenaline alone.

Goldsmith [552] and her associates have found that asthmatics have low blood vitamin C levels, and that they are unable to maintain a level of 1.0 mg. per 100 c.c. even with large supplements. This is interpreted to mean that there is an increased requirement of vitamin C in asthma. It is possible that the laboured breathing involves additional muscular effort and might raise the metabolic rate, which increases the vitamin C requirements. Possibly an increased need is related to the allergic reaction although there is no proof of this. In two of the patients studied there

appeared to be some relationship between the amount of vitamin C in the blood and the frequency and severity of the asthmatic attacks. These patients were much improved after saturation with vitamin C.

Hunt [772] administered 100 mg. of vitamin C a day to twenty-five asthma patients, but he was unable to observe any diminution in the severity or incidence of the attacks. Five patients obtained no relief after the intravenous injection of 500 mg. to 800 mg. of the vitamin, nor had the treatment any effect in diminishing the amount of adrenaline required.

Holmes and Alexander [681] claim that vitamin C has a beneficial effect in hay fever, and that in doses of 200 to 500 mg. daily it produced "remarkable improvement" in twenty-five patients with ragweed hay fever. The work was not controlled. Other observers have failed to confirm observations [733, 1089].

Gastro-Intestinal Diseases. The gastric absorption of vitamin C and its inadequate absorption in certain gastro-intestinal conditions has been mentioned (p. 498). Many workers have shown that a very low level of vitamin C nutrition exists in patients suffering from gastric and duodenal ulcers, and from hæmatemesis and melæna which occur as complications [477-481, 484-487]. Thus Ludden, Flexner and Wright [743] found that in twenty-three patients with gastric lesions only one had a normal vitamin C reserve; one had frank scurvy. The reason for these low levels is partly because of a low intake, since peptic ulcer diets consisting as they do of milk, eggs, slops, white bread and fish contain very little vitamin C, and partly because of defective absorption. Rao [774], however, examined a number of peptic ulcer patients in India, but failed to find any evidence of vitamin C deficiency as judged by studies on the urinary excretion and blood level of the vitamin. In some cases peptic ulcer patients have been reported as being almost scorbutic after long continued dieting with food-stuffs containing very little vitamin C [481, 775]. Platt [775], for example, describes four cases of adult scurvy resulting from strict adherence to special diets. The patients suffered from hæmaturia, purpuric rashes, epistaxis, and bleeding gums—all typical scorbutic symptoms—which cleared up after administering vitamin C.

According to the figures of Warren [484a] and his colleagues a person weighing 150 lbs. requires about 70 mg. of vitamin C a day, whereas the ulcer patient on the first week Sippy diet receives approximately 5 mg., and 15 mg., a day during the fourth week. The inadequacy of the Sippy diet in vitamin C is shown by the relatively large amount of vitamin C, about 1,800 mg., required to saturate a patient on the diet for three weeks.

In view of the importance of vitamin C in wound healing (p. 465), patients with peptic ulcer and hæmatemesis or melæna of gastric origin should receive additional vitamin C to assist the healing process, *e.g.*, up to 50 mg. twice daily after saturation with the vitamin. If absorption is considered to be defective large doses can be given by mouth or smaller doses parenterally. Hunt [77] has described post-mortems on twenty-eight patients dying several days after perforation of a peptic ulcer, or after operations on the stomach. In those patients with a low level of vitamin C nutrition wound infection was common, and collagen formation, which is

essential for the repair of a wound or the healing of an ulcer, was either poor or absent. Microscopically some of the wounds were like those of scorbutic guinea-pigs. There is no reason to believe that vitamin C deficiency is aetiologicaly related to peptic ulceration, as was suggested by the work of Smith and McConkey [74], who noted that guinea-pigs suffering from prolonged scurvy tend to develop peptic ulcers, an observation confirmed by Roe [806] and his co-workers.

Several workers have reported an improvement in peptic ulcer patients and cases of hæmatemesis treated with vitamin C. While such treatment is rational and is in agreement with our knowledge on the physiological functions of vitamin C, it must be admitted that the cases studied have not been numerous and they have not been controlled. Singer [776] describes twenty-five cases of peptic ulcer, twenty-one of which showed signs of vitamin C deficiency. The administration of the vitamin in doses of 800 to 2,000 mg. was stated to have a distinctly favourable effect in promoting the healing of the ulcers. Demole and Guye [777] treated thirteen cases of peptic ulcer with vitamin C given parenterally in doses of 175 mg. in addition to histidine, a remedy that has now been shown to be quite valueless in the treatment of peptic ulcer. A total of twelve to eighteen injections was given; eight cases were cured and two improved.

Schnetz [476] states that he has observed subjective signs of vitamin C deficiency in other digestive disorders, such as duodenitis, enteritis, achlorhydria, diseases of the gall bladder, and after gastrectomy. The diets in these conditions are practically devoid of vitamin C. He observed a general improvement in thirty patients after the administration of vitamin C. Abt, Chinn and Farmer [478] have shown that vitamin C deficiency exists in most patients with achlorhydria. It is possible that hydrochloric acid is needed for the absorption of the vitamin by mouth, although alkali and buffer therapy in the treatment of gastric ulcer does not seem to interfere with its absorption [882]. The absorption of vitamin C may be grossly defective in sprue. A case is described by Mohr [520] in which the intravenous administration of 400 to 500 mg. of vitamin C for nineteen days was necessary to achieve saturation. It was subsequently impossible to maintain oral treatment owing to the atrophic condition of the gut.

Obstetrics. The increased need of vitamin C in pregnancy and during the period of lactation is discussed on p. 519. Roughly speaking the pregnant woman requires from two and a half to three times the minimal intake of an average woman, that is from 75 to 100 mg. a day; the nursing mother requires from 100 to 150 mg. a day. Normally these quantities can be supplied by the food if the mother is on an adequate diet.

Recent studies have shown that there is some degree of vitamin C subnutrition in pregnant women in the British Isles [998]. This has been attributed to war-time diet. In 1938, before the war, Elmby and Christensen [421] found that the serum vitamin C was diminished in pregnancy and they concluded that the winter diet from October to April supplied insufficient vitamin C. This was confirmed by Craig and his co-workers [998], who have also noted a high incidence of spongy gums in pregnant

women in war time. It is not certain whether the latter is caused by vitamin C deficiency.

It is well recognized that the diet of the pregnant woman is very important. According to Williams and Fralin [874] only two per cent. of women consume an adequate diet in pregnancy. Ebbs, Tisdall and Scott [488] observed an increased incidence of abortion in patients on poor diets. In early pregnancy, morning sickness and vomiting may result in conditioned vitamin C deficiency. In an experiment on over 5,000 pregnant women carried out under the auspices of the People's League of Health between 1988 and 1989, it was found that the administration of supplements of vitamin C, halibut liver oil, the vitamin B complex and minerals, resulted in a diminished incidence of toxæmia of pregnancy and prematurity [789].

Javert and Stander [989] believe that lack of vitamins C and K may be a factor in the pathogenesis of threatened and spontaneous abortion and antepartum bleeding. They find that plasma vitamin C decreases in normal pregnancy from non-pregnant values of 1.1 mg. per cent. to 0.85 or 0.4 mg. at term. Of seventy-nine patients with threatened, spontaneous or habitual abortion, deficiency in vitamin C was found in sixty-nine per cent., and combined vitamin C and K deficiency was present in sixty-one per cent. These patients usually complained of skin ecchymoses, epistaxis, bleeding gums and vaginal bleeding. Thirty-three patients with threatened abortion or a history of previous abortions or three or more consecutive abortions were given vitamins C, E and K, minerals and trace elements (iron, copper, cobalt, nickel, zinc and manganese) and progesterone. The abortion incidence was twenty, nil and seven per cent. for each group, whereas in a control group of forty-six patients it was one hundred per cent. So many preparations were administered in this study that it is difficult to ascribe the lowered incidence of abortion to any one of them. Javert and Stander observed that several newborn infants with cord blood values of 0.0 and 0.8 mg. of vitamin C per cent. developed hæmorrhagic disease. Since hypoprothrombinæmia is almost universal at birth, whereas the vitamin C blood level is usually normal, Javert and Stander believe that a deficiency of both vitamins C and K is necessary for the development of the hæmorrhagic disease of the newborn.

Several reports have appeared suggesting that vitamin C given prophylactically prevents threatened abortion in women who have aborted previously. Ley [781] saturated ten women who had two or more abortions with vitamin C early in pregnancy, and gave maintenance doses by mouth until the end of the sixth or seventh month. All ten patients gave birth to a living child. Others have reported similar cases treated successfully with vitamin C [782, 788]. The facts are unconvincing because of the lack of adequate control, and because it is well known that women who have previously aborted often become pregnant again and give birth to healthy living children without any specific form of treatment. The subject of vitamin E and abortion is discussed on p. 748.

Prolonged hyperemesis gravidarum is frequently accompanied by

varying types of vitamin deficiency (*cf.* vitamin B₁, p. 209). In this condition adequate supplements of vitamin C and the B complex should be given. It has been suggested that vitamin C deficiency may be the cause of hyperemesis gravidarum [784], but it is more likely that the latter causes a conditioned vitamin C deficiency.

Käppeli [780] has carried out some remarkable pharmacological experiments on the effect of vitamin C in labour. He states that in the isolated guinea-pig uterus the addition of vitamin C to the perfusing liquid causes increased uterine tone, reinforces the effect of pituitary extract, and in large doses produces a slow, steadily increasing uterine contraction which either becomes rhythmic or passes into tetany. The effect of vitamin C on the human gravid uterus is variable. It has an oxytocic action according to Käppeli, but either a contraction passing into tetany is produced, or the uterus relaxes. On the other hand, it strengthens and prolongs the uterine contractions caused by pituitary extract. The effect was observed of giving vitamin C in childbirth, either orally or intramuscularly in doses of 100 to 250 mg. Here its action is not so variable as when given intravenously. After five to fifteen minutes it exerts a definite oxytocic action, intensifying the labour pains, and its effect lasts for forty-five to sixty minutes. It does not induce labour and appears to have very little effect on post-partum hæmorrhage. The work is of considerable interest, but it has not yet been confirmed.

Vitamin C in Surgery. Wound Healing. The importance of vitamin C in the healing of wounds has been previously mentioned (p. 465). Hartzell [845], Lund [786], Bartlett [787] and his collaborators, and others [778, 788], have shown that the majority of patients are suffering from vitamin C deficiency when they come to operation, and that for some days after operation there is a considerable drop of thirty to fifty per cent. in the vitamin C excretion and blood level.

The deficiency of vitamin C is even more severe in patients undergoing operations for gastric lesions because of the dietary restrictions before operation. Lund [786] found that a number of gastric cases coming to operation had only twenty to fifty per cent. of normal vitamin C reserves, and some were bordering on scurvy. When non-radical operations were performed more complications and deaths occurred in the patients with low vitamin C reserves than in those with normal or high reserves.

This fall in the vitamin C level cannot be accounted for solely on the basis of a restricted intake of the vitamin just after operation because the fall is too sudden. According to Bartlett [787] and his colleagues there is some evidence that the magnitude of the surgical procedure is related to the extent and duration of the post-operative vitamin C deficiency. An intravenous dose of 1,000 mg. of vitamin C disappears from the blood stream far more rapidly after operation than before. This increased need for vitamin C in the surgical patient is due to its utilization in the process of tissue repair and wound healing (p. 465).

Bartlett and his colleagues [852] have also shown that a sufficient depletion of vitamin C will produce a decreased vitamin C content and decreased tensile strength in healing wounds in the skin and fascia of human beings. According to them a fasting plasma level of 0.20 mg. of

vitamin C per 100 c.c. must be reached for this to occur, although Bourne [925] has been unable to confirm this. In the presence of adequate vitamin C the vitamin C content of healing fascial scars, compared with control biopsies is much greater than that of healing skin scars.

Vitamin C deficiency is one of the causes of wound eventration and disruption, which has been reduced by seventy-five per cent. in a series of cases studied by Hunt [77], in which the routine administration of vitamin C was adopted for all major operations. In the same series leakage from suture lines occurred only once in a large number of operations conducted over a period of thirty months. When wounds failed to heal in spite of an adequate supply of the vitamin, there was always gross local infection, a hæmatoma, or ischæmic necrosis. From studies on vitamin C and infection it is reasonable to assume that the healing of infected lesions would be assisted by saturating the patient with vitamin C. Hunt [77] examined histological material from abdominal incisions, gastro-intestinal anastomoses and stomata, sutured duodenal ulcers, a repaired intestinal rupture and some other specimens from twenty-eight patients that had undergone operation, but had succumbed after periods varying from a few days to three weeks. In all those cases in which collagen was poorly formed and cellular proliferation scanty the intake of vitamin C had been insufficient (Figs. 153 and 155). In five of these cases abdominal disruption had occurred after operation in the absence of gross infection, and three had also leaked at the suture line without local necrosis. Microscopically, their wounds were very similar to those of sub-scurvy guinea-pigs (Figs. 116 to 119 and 155). Histological examination of the wounds of those patients who had received vitamin C after operation revealed that collagen formation and cellular proliferation were taking place normally. Figs. 152 and 153 show cross-sections of sutured skin incisions in two of Hunt's cases. The former is from a patient who died twelve days after operation and who had previously received plenty of vitamin C; there is exact apposition of the cut edges and little precollagen remains. In the latter, from a patient who died thirteen days after operation, but who had previously received no vitamin C and had little reserve of the vitamin in his body, it is seen that the opposed edges of the corium are stretched apart and the gap filled by an immature precollagenous scar. Hunt also believes that the absorption of catgut ligatures may be delayed (Fig. 155), and that post-operative peritonitis is more common in patients with inadequate reserves of vitamin C. Kraybill [1051] observed a zero blood vitamin C in seven cases of wound disruption after abdominal operations.

Lund and Crandon [818] studied the effect of pre-operative diets and plasma vitamin C levels on the post-operative healing of abdominal wounds. While they agree with Hunt that post-operative herniæ occur more readily in patients whose vitamin C intake has been low, they believe that mechanical factors are often more important in wound disruption than vitamin C deficiency, although this can be a cause. Other causes are hypoproteinæmia and infection. Despite a low vitamin C level in eight patients vitamin C deficiency played no part in post-operative wound disruption. Lund and Crandon suggest that the problem of wound disruption is largely associated with imperfect suturing. They do suggest,

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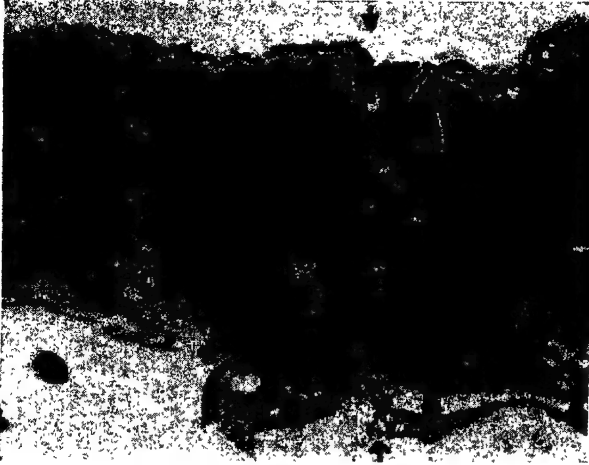


FIG. 152. Vitamin C and Wound Healing. Cross section of a sutured skin incision of a patient given 2,600 mg. of vitamin C in divided doses after operation for perforated duodenal ulcer. Stain, silver impregnation. There is exact apposition of cut edges with good collagen formation. Little precollagen remains.



FIG. 153. Vitamin C and Wound Healing. Cross section of a sutured skin incision of a patient grossly deficient in vitamin C. Stain, silver impregnation. The opposed edges of the corium are stretched apart and the gap filled with a mass of proliferative fibroblasts producing immature precollagen only. In other regions where the scar was more stretched the gap was filled with oedematous granulation tissue with little precollagen and no collagen.

VITAMIN C AND WOUND HEALING



FIG. 154. Vitamin C and Wound Healing. Control. Cross section of the gastric incision of a control normal guinea-pig killed twenty-one days after operation. Stain, silver impregnation. Note the uniform mature healing, and disappearance of catgut sutures (which disappeared on the seventeenth day). The epithelium is well healed and the only signs of the initial incision are the fusion of the layers of the muscularis and the substitution of the muscle by fibrous tissue.



FIG. 155. Vitamin C and Wound Healing. Subcorbutic guinea-pig. Cross section of the gastric incision of a subcorbutic guinea-pig killed twenty-one days after operation. Stain, silver impregnation. Note irregular delayed healing with a catgut ligature still not absorbed or extruded, and precollagen forming the intercellular substance in the subserous part of the scar.

however, that the vitamin C reserves of surgical patients should not fall too low. They believe that a daily intake of 20 mg. of vitamin C is sufficient for wounds to heal easily and well. Pijoan and Lozner [1075] consider that a daily dietary intake of 12 to 25 mg. of vitamin C, which is sufficient to maintain a level of 25 mg. per 100 gm. in the white cell layer (p. 552), is sufficient to produce adequate wound healing and collagen formation. They base this view on observations made on a patient whose daily intake of vitamin C for twenty months averaged 16 mg. The plasma vitamin C was never more than 0.2 mg. per 100 c.c. An experimental wound was made in the back and ten days afterwards a biopsy revealed normal healing with ample intercellular substance and capillary formation.

According to Bartlett and his co-workers [852] normal wound healing may result, in spite of a low content of vitamin C in the plasma before operation, if adequate vitamin C is given post-operatively. They have also shown that focal infection distant from the healing scar does not alter its vitamin C content or its tensile strength [1081]. Local wound sepsis, however, reduces the tensile strength of a wound and interferes with its normal healing. Apparently the local use of sulphanilamide does not reduce the tensile strength of a wound scar nor does it retard healing. Analysis of scar tissue also reveals that sulphanilamide applied locally does not diminish its vitamin C content or increase the need of vitamin C in the production of a normal strong cicatrix. In the presence of considerable vitamin C deficiency local sulphanilamide does not promote wound healing nor does it adequately control wound infection. This observation is important. It shows the futility of dusting wounds with sulphonamides if the vitamin C nutrition of the patient is neglected. Bartlett and his co-workers [1081] noted that in scorbutic animals the production of focal walled-off abscesses distant from the operative scar was followed by ultimate infection of the scar with the same organism. They call attention to the possibility that maximal tissue saturation with vitamin C may increase tissue resistance to infection as well as promote optimal conditions for wound healing.

From a study of thirty cases before and after operation Wildbolz [791] concluded that there was little evidence that vitamin C deficiency had any relation to post-operative complications. The administration of vitamin C to all operation cases during the course of a year reduced the incidence of post-operative complications from 24.2 to 19.7 per cent., but this reduction was not considered to be statistically significant.

The necessity for the administration of vitamin C both before and after operation is clear. 1,000 mg. given daily for several days should be enough to saturate even a grossly deficient patient, although Wolfer and Hoebel [75] prefer to give this dosage for nine to ten days. Griessmann [790] gives 300 mg. to 500 mg. intravenously three to four hours before operation. The patient should then be maintained on high doses of the vitamin, *e.g.*, from 800 mg. to 500 mg. a day in divided doses to minimize the rapid excretion through the kidney. It is possible that much smaller doses than this will suffice—possibly 100 mg. a day as advised by Hunt [77]—but as the requirements of the surgical patient are unknown and depend upon so many factors it is advisable to give the maximum

amount. Certainly cases with a history of a low vitamin C intake, infection, chronic gastro-intestinal disease, new growth, and achlorhydria require considerably increased amounts. It is suggested that it should be given with hydrochloric acid if achlorhydria is present or suspected, as in carcinoma of the stomach and ulcerative colitis. Evidence suggests that it is poorly absorbed by mouth in this condition (p. 499), and it should either be given parenterally or in large quantities orally. Vitamin C in the form of the sodium salt may be given in intravenous drip infusions in a dosage of 200 mg. to the pint [77].

It is suggested that the administration of vitamin C to surgical patients should be particularly considered under the following circumstances [77]: (a) when clean and quick healing of a wound is desired; (b) in major operations; (c) when a hollow viscus has been opened; (d) when post-operative complications are anticipated; (e) when there is a history of insufficient intake of vitamin C; (f) in all cases of serious injury; (g) in patients with a history of vomiting over long periods; (h) in cases of obstructive gastro-intestinal lesions and hypermotility of the small intestine; (i) in syphilitics and alcoholics; and (j) in patients receiving fluids such as glucose or saline intravenously or per rectum over a prolonged period.

The importance of an adequate intake of vitamin C for the healing of wounds is recognized by the Medical Division of the National Research Council, U.S.A., who recommend a daily oral intake of 75 mg. in all wounded and burnt men in the Services until recovery is complete [1080].

According to Evans [1082] skin grafts do not take readily in subjects with a low level of vitamin C nutrition.

Vitamin C and Sulphonamides. Ruskin [1078] reports the successful use of sulphanilamide ascorbate and sulphathiazole ascorbate in the healing of chronic wounds, particularly in unhealed cavities following mastoidectomy, and in ears with chronic suppurative otitis media. The cavity is irrigated and packed with the sulphonamide ascorbate, left for a week and then swabbed out. The results were stated to be good, but since the observations were not controlled and excellent results have been obtained with sulphonamides alone, it is difficult to see what advantages a sulphonamide ascorbate has over the sulphonamide itself. As the reaction of such a compound is acid it might even retard wound healing. Williams and Bissell [1085] have shown that vitamin C produces no definite benefit when applied locally to wounds in rats.

Burns. Vitamin C has been used in conjunction with adrenal cortex extract in the treatment of shock following burns, but it is difficult to tell whether the beneficial results that have been claimed for the treatment are due to the vitamin C or the adrenal cortex extract, or both, or to spontaneous recovery [792-794]. Einhauser [792] describes a case of a boy with second and third degree burns that covered fifty-five per cent. of the body surface, who was treated successfully with adrenal cortex extract and vitamin C in doses of 500 to 750 mg. a day. Histological studies by Urechia [798] and his colleagues on ninety-one rabbits and two human beings, who succumbed to scalds with boiling water, showed that the adrenals were a focus of an energetic reaction; in both animals and

human beings the level of vitamin C in the blood fell and increased in the adrenals.

Vitamin C is certainly required for the formation of fresh epithelial tissue in burns. As in the case of wound healing a certain optimal level of vitamin C is needed and no better results are to be expected by exceeding this.

Shock. Loss of hæmoglobin in acute hæmorrhage and circulatory failure in shock both lead to a considerable diminution in the oxygen supply to the tissues. In order to improve the transfer of oxygen from the blood to the tissues in cases of hæmorrhage and circulatory failure, Stewart and his associates [795] suggested the intravenous injection of vitamin C. Five control cats were bled until the blood volume was reduced to half; the blood pressure fell considerably and the animals died within an hour. Sixteen other cats were treated similarly, but they also received neutralized vitamin C (sodium ascorbate) intravenously in doses from 200 mg. per kg. of body weight to a dose of 2 gm. In all cases the blood pressure rose and the survival period was much longer than in the control animals. In those animals that survived, a striking feature was the rapid reappearance of good pulse pressure and improvement in respiration after the injection of vitamin C. The work of Stewart and his colleagues certainly suggests the use of vitamin C intravenously in cases of accidents and injuries involving hæmorrhage, that cannot be given an immediate transfusion of blood or plasma, *e.g.*, war and air raid casualties. The suggested dosage in such cases is 3 gm. of sodium ascorbate in 10 c.c. of solvent. This could easily be given intravenously after identifying a vein and cleaning up the skin. The observations of Lucas confirm Stewart's suggestion that vitamin C secures a more adequate supply of oxygen for the tissues. Lucas [804] has shown that vitamin C administered intraperitoneally increases the resistance of mice and rats to low oxygen tensions. It has also been demonstrated that the addition of low concentrations of vitamin C increases the rate of respiration of isolated liver tissue [845].

Ungar [1038], using a standard traumatizing technique in guinea-pigs, rats and mice, has shown that the injection of vitamin C within fifteen minutes after trauma reduces post-traumatic mortality in these animals. The minimum effective dose was 100 mg. per kilogram of body weight. This effect is independent of the vitamin action of vitamin C, as it can be produced after oxidation; moreover rats and mice, the experimental animals used, do not suffer from vitamin C deficiency as they synthesize their own vitamin C. If these results are applicable to the treatment of human traumatic and surgical shock a comparable dose would be 7 grams.

Further experiments on vitamin C and shock were made by McDevitt and her co-workers [1034]. They showed that scorbutic guinea-pigs are more susceptible to shock than normal animals, and that repeated trauma in animals with a normal intake of vitamin C "conditions" them to traumatic shock. It was found that massive doses of vitamin C immediately following trauma increase the survival time of animals, but do not lower the mortality rate. McDevitt also studied the post-operative vitamin C plasma levels of patients submitted to surgery; of eleven cases only six had normal values. If these data can be transferred from guinea-pig

experiments to human surgical and traumatic shock, the addition of liberal amounts of vitamin C (*e.g.*, 1 gm. or more) to blood or plasma might increase the therapeutic effectiveness of these agents, which are the sheet anchor in the treatment of shock.

Anæsthesia. Clinical studies have not been made, but animal experiments suggest that tolerance to anæsthetics may be increased by administering vitamin C. The urinary excretion of vitamin C is increased in the dog, rat and guinea-pig after ether anæsthesia, and analysis of the adrenals, kidneys, liver and ovaries shows a fall in the vitamin C content of these organs after ether, chloroform or ethyl chloride anæsthesia [796, 218]. Guinea-pigs treated with 50 mg. to 100 mg. of vitamin C show an increased tolerance to anæsthetics; they can withstand anæsthesia for a longer period than control animals that have not received the vitamin [218].

Beyer, Stutzman and Hafford [1044] have shown that after anæsthesia with cyclopropane, ether, divinyl ether (vinesthine) and chloroform, the plasma vitamin C rises for the first seven hours, subsequently falling in twenty-four hours below the original level. This would account for the increased excretion of vitamin C after anæsthesia found by others. Beyer and his co-workers have also shown that in animals suffering from vitamin C deficiency anæsthesia can be induced more rapidly, the animals are more depressed by a given dose, and they recover more slowly than healthy control animals treated with 80 mg. of vitamin C daily. If these data can be transferred to clinical anæsthesia, they suggest that large doses of vitamin C might be helpful before an anæsthetic is administered.

The importance of vitamin C in the healing of fractures is discussed on p. 464.

Psychiatry. In view of the dietetic idiosyncrasies and increased physical activity of some mental patients and the low vitamin C content of most institutional diets it is not surprising that a considerable degree of vitamin C deficiency has been found in such patients [495-508]. Some investigators have found low vitamin C levels in patients suffering from alcoholic and senile psychoses [495, 499, 508]. Barahal and Priestman [799] state that there is undoubtedly a deficiency of vitamin C in most psychiatric cases, regardless of the diagnostic grouping, including senile and alcoholic psychoses, in which it is conditioned by deficient intake (*cf.* p. 209). There is no evidence of any direct causal relationship between vitamin C deficiency and mental disorder. If a deficiency is found, it is usually due to malnutrition. One would expect, however, by improving the nutritional status of a patient to improve the general condition and even mental outlook of the patient.

Alexander [495] suggests that the low levels of vitamin C found in patients with alcoholic psychoses may be a factor in the production of subdural hæmatomata, since their frequency in such patients cannot be accounted for by trauma alone. It is very unlikely, however, that vitamin C deficiency is a cause of hæmorrhage (p. 541). In some mental disorders metabolic, toxic and endocrine factors are of importance, and in these it would seem rational to ensure an adequate intake of vitamin C. Minski and Constantine [797] found no correlation between the mental condition and the level of vitamin C in psychotic and psychopathic patients, nor did

they observe any material change in the mental state of patients who had been treated with vitamin C. Stotz [842] failed to observe any signs of vitamin C deficiency in patients with schizophrenia. Merland and Ollivier [798] claim that the administration of the vitamin to patients with mental confusion brought about some improvement in their condition in nineteen out of twenty-seven cases. The mental confusion was less noticeable after treatment; after the fifth injection their orientation was said to have improved, their senses were clearer, appetite returned, digestion was improved, and in some patients there was an increase in weight. The same authors describe the treatment of six cases of acute psychotic encephalitis with uræmia by intramuscular injections of 100 mg. of vitamin C a day. The temperature and the blood urea fell, the patients were less restless and slept better, appetite returned and the general condition improved. As in so many clinical investigations controls were not observed and the possibility of spontaneous improvement cannot be excluded.

Hoff and Shaby [800] give an account of the treatment of nine cases of acute confusion developing during typhoid fever, and after difficult labour, with adrenal cortex extract and vitamin C (dose not stated). No other treatment was given. In each case the patients began to clear mentally, were easier to manage, slept better and were no longer disoriented. Other confusional states of indefinite origin did not respond.

Vitamin C as Diuretic. Abbasy [801] has observed that vitamin C in doses of 700 mg. has a specific diuretic effect. This was noted during investigations on the excretion of vitamin C after giving test doses (p. 544), and confirmed by using control subjects. It is claimed that the diuretic property of vitamin C may be of some use in cases where a slow and progressive dehydration of the body is desired, particularly as it is safe and unlike mercury diuretics does not damage the kidney. Evans [802] studied the diuretic effect of vitamin C in eight cases of heart failure and one of cedema of unknown origin. In doses of 500 mg. to 800 mg. a day it produced a diuresis in all patients, as judged by excess of urinary output over fluid intake; its diuretic effect was greater than that of digitalis but less than that of theobromine, diuretin and ammonium chloride. The diuretic effect of vitamin C has also been observed by Lueg and Hammann [808], who state that it can be used to reinforce the mercurial diuretics, such as mersalyl, and actually increases their tolerance, since toxic effects on the kidney and liver are not so frequently observed when they are given with vitamin C.

Shaffer [1085] noted that vitamin C in doses of 500 mg. produced a diuresis in patients with cardiac decompensation. The actual increase on a standard fluid intake of 1,500 c.c. was from 250 to 1,000 c.c. in seventy-two hours. When given intravenously, vitamin C had no appreciable effect, possibly due to its rapid elimination by the kidney. In combination with the diuretic mercupurin there was a relatively large diuresis of from a half to two and a half times that produced by mercupurin alone.

Effort Syndrome. Croft, Jones and Richter [1086] found that neither vitamin B₁ nor vitamin C deficiency is a significant factor in producing fatigue and other symptoms in patients with the effort syndrome. This

is characterized by fatigue, effort intolerance, breathlessness, palpitation and præcordial pain.

Detoxicating Action of Vitamin C. See pp. 482 to 488.

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CHAPTER VII

VITAMIN D

THE ANTIRACHITIC OR CALCIFYING VITAMIN

SEVERAL different substances are now known to be antirachitic : the term vitamin D is used to embrace them all. The most important are :—

Vitamin D₂ or Calciferol. This does not occur naturally but is manufactured artificially by irradiating or “activating” the vegetable sterol ergosterol. Viosterol is a name chiefly used in the United States for unpurified irradiated ergosterol : it has little meaning, as the amount of calciferol in viosterol varies greatly, according to the method of irradiation employed.

Vitamin D₃. This is the most important naturally occurring vitamin, being formed in the skin by the action of the sun on the animal sterol 7-dehydrocholesterol.

Vitamin D₁ is a name which is no longer used, having been given to a substance which was later found to be an addition compound of calciferol and lumisterol.

HISTORY

The early history of vitamin D is the history of rickets. It is a depressing history : a history of perfect clinical observation on the cure of the disease being forgotten again and again for a century and a half.

Rickets suddenly became recognized as a definite disease by medical writers in the last half of the seventeenth century ; the poor had probably been familiar with it for many years. The name rickets, according to Skeat [1], is an old English word : the adjective rachitic was forced into our language by the mistaken desire of lovers of the classics to give rickets a Greek derivation.

The first description of rickets is given by Daniel Whistler, who, in 1645, published his “*De Morbo puerili Anglorum, quam patrio idiomate indiginæ vocant ‘The Rickets.’*” This was a thesis presented for his Doctorate of Medicine at Leyden, which has led some to say he was German. He was, however, an Englishman educated at Thame and Merton College, Oxford. His description of rickets, written at the age of twenty-five, was followed four years later by another by Arnold Boate, and it was not until 1650 that Glisson [2], a Cambridge man, published his account, which for some obscure reason is generally said to be the first. A few much earlier descriptions of cases of what were probably rickets or its adult equivalent, osteomalacia, have been collected [99, 101], though these were not recognized as an individual disease, but reported as curiosities.

The eighteenth century added little to the history of rickets until towards its end when cod liver oil was first used in medicine, though apparently in Scotland and Northern Europe cod liver oil had been popular for many years with the peasants as a cure for rickets and other diseases.

In 1782 Dr. Robert Darley [8] wrote to Dr. Thomas Percival an account of his use of cod liver oil, which was so highly successful that the poor clamoured for it, though its smell and taste were loathsome as it was made by "heaping together the livers of the fish, from which, by gentle putrefaction the oil flows very plentifully." Percival [8] recommended peppermint to conceal the taste—advice which a century and a half of further experience has not bettered. But it must be admitted that the earliest account of the value of cod liver oil did not stress its value in rickets: in fact, only two children were reported, the other cases being arthritis or rheumatism.

After this the use of cod liver oil for rickets seems to have spread rapidly in Germany and Holland and from there to France. By the middle of the last century Trousseau [4] was teaching that cod liver oil was the well known and perfect cure for rickets, or when this was too expensive, large quantities of the best fresh butter—suitably concealed in a mixture so as not to shake the confidence of the patient by prescribing such a simple remedy. He also realized the uselessness of vegetable oils, the value of sun, the drawbacks of cereals, and identified osteomalacia as adult rickets.

The last century had also gone far in elucidating the nature of rickets by animal experiments. Jules Guérin [5] in 1838 had produced rickets in puppies to support his theory that rickets was due to the wrong food, and Bland Sutton, fifty years later, had used cod liver oil, milk, and crushed bone to cure rickets in lion cubs at the Zoo.

At the beginning of the present century all this brilliant clinical observation appears to have been largely forgotten, even though Trousseau's book had been translated into English by the New Sydenham Society. In 1912 Sir William Osler was still only vaguely mentioning cod liver oil as useful in rickets. Indeed, there was amazing confusion on the subject. There were three outstanding theories.

One theory was that it was a chronic infective condition like tuberculosis, this being still widely believed on the Continent as late as 1919.

The second theory was Hanseemann's "Domestication Theory" which was upheld by the "Glasgow School," so called from the observations of Fergusson and Findlay [7] on rickets in Glasgow in 1918. The theory is well summed up by Hutchison and Shah [8] in their observations on the effect of Purdah in India in 1922: "The most important ætiological factor in the production of rickets is lack of fresh air, sunlight and exercise." But sunlight was never stressed as the one important factor in fresh air and exercise, though Palm [9] had emphasized this in 1890.

Thirdly, the theory was gaining ground that rickets was a deficiency disease. This was due to the work of Edward Mellanby [10], who by 1918 had produced experimental rickets in puppies by feeding them on diets which were deficient in some factor found in certain animal fats. At first this factor was thought to be the same as the growth factor described in 1918 by McCollum and Davis [11] and the "fat soluble A factor," which was necessary for the protection of the eyes—the antixerophthalmic factor—described in 1917 by McCollum and Simmonds [12].

But further work showed that the antirachitic factor and the anti-

xerophthalmic factor were different. They did not always occur in the same proportion in all fats tested ; one fat might give little protection against xerophthalmia but give good protection against rickets, while another fat might have the opposite effects. It was also found that heat and oxidation destroyed more of the antixerophthalmic than the antirachitic factor.



FIG. 156. Rickets in Vienna in the post-War famine of 1920. The children, six years of age, show severe rachitic deformities compared with the normally grown child of the same age in the centre. (See also Fig. 165.)

The importance of calcium and phosphorus in relation to rickets was shown by McCollum when extending Mellanby's work to rats, animals which only develop rickets if these two elements are badly balanced in the diet.

In 1919 Chick and her co-workers went to study rickets in Vienna during the post-War famine. The work was originally undertaken to follow up the clinical possibilities of Mellanby's work. It was extended to include the effect of sunlight, and exposure to the radiations from the mercury-vapour quartz

lamp, because of the observations of Huldshinsky [18] and others, who had definitely proved that rickets was curable by these forms of light. Chick's final report in 1928 [14] showed that both cod liver oil and sunlight, or the mercury-vapour quartz lamp, cured rickets ; but improved hygiene with neither cod liver oil nor sunlight was useless. In fact, the dietetic theory of rickets was completely confirmed, and the teachings of the Glasgow school in so far as they taught or implied the value of sunlight.

The end of the complicated story of rickets, food, and sunlight was unravelled by the work of Hume [15] and many others ; though to the last there were unexpected complications, as when rats did not develop rickets because they were themselves supplementing their deficient diets by eating irradiated sawdust in their cages. Many experiments proved that ultra-violet irradiation of an animal or of food produced the anti-rachitic substance, the ultra-violet light activating a substance—"the provitamin"—found only in the unsaponifiable fraction of fat.

So it was finally shown that animals can either make their own vitamin

D by the aid of the ultra-violet rays of the sun, or they can get it by eating other animals which have themselves already made it.

CHEMISTRY OF VITAMIN D

All the forms of vitamin D so far investigated are derived from sterols, generally by photochemical reactions. They are chemically, but not apparently physiologically, related to the sex hormones, the bufotoxin of toad venom, the digitalis and strophanthus alcohols, and the carcinogenic hydrocarbons [6]. The chemistry of the sterols is extremely complex: the following account of the formation and properties of vitamin D₂ and vitamin D₃ is largely taken from the excellent review and papers by Bills [16]. For an account of the early work on the chemistry of vitamin D₂ and its preparation in a pure form by English and German workers in the same year (1931), the reader should consult the Medical Research Council's "Vitamins: A Survey of Present Knowledge," published in 1932.

Vitamin D₂, or calciferol, was the first of the D vitamins to be fully investigated, though as will be seen when the physiology of vitamin D is discussed, it is an entirely artificial product made from ergosterol—a sterol only found in plants, especially fungi. It does not occur naturally, as opposed to vitamin D₃, which is the vitamin formed and used by animals [17] and the only one as yet identified in fish liver oils [75].

Vitamin D₂ is made by exposing ergosterol to the action of ultra-violet light. The absorption spectrum of ergosterol gives bands of maximum intensity at 260, 270, 282, and 293.5 millimicrons. As would be expected from this it is wavelengths of about 230 to 305 millimicrons which affect the change of ergosterol to calciferol. But calciferol is not the final product which can be produced by irradiating ergosterol: for calciferol itself is changed by irradiation, especially by wavelengths shorter than 270 millimicrons.

Ergosterol can be irradiated either as a solid or in solution: the latter is the only satisfactory way, the products of irradiation on the outside of the solid ergosterol apparently protecting the rest of it from the effects of irradiation.

The solvent is important; for instance, if alcohol is used it is difficult to avoid over-irradiation, with the result that vitamin D₂ or calciferol is destroyed with the production of the toxic toxisterol in its place. Ether, on the other hand, makes it relatively easy to control the irradiation and so to form calciferol with little or no toxisterol. This effect of the solvent is called the specific solvent effect; it is not clearly understood.

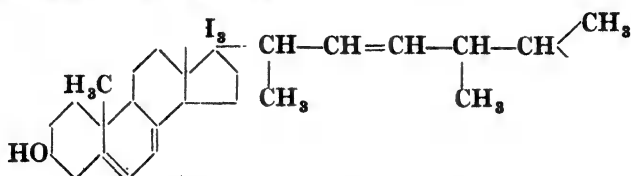
The change from ergosterol to calciferol is purely a photochemical one, involving only a rearrangement of the molecular structure. It is almost unaffected by temperature, though the presence of more than minute quantities of oxygen should be avoided.

The usually accepted series of reactions which occur when ergosterol is irradiated are: ergosterol, lumisterol, tachysterol, calciferol, toxisterol (substance 248), suprasterol I and suprasterol II.

It must be remembered, however, that all these reactions are going on at the same time. It is necessary to stop irradiation when only about half

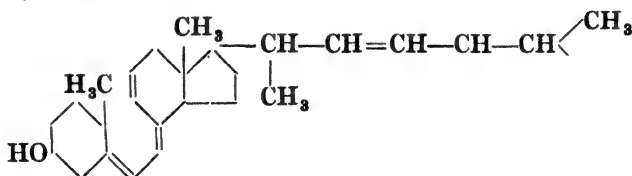
the ergosterol has changed to calciferol, or the continued irradiation will destroy the calciferol which has already been formed. The result is that at the end of the irradiation large amounts of lumisterol and tachysterol with traces of toxisterol are still present. In the Whittier and other processes these drawbacks of making calciferol by irradiation are said to be avoided by activating ergosterol in various ways such as vaporizing it and then exposing it to an electrical discharge [229].

Ergosterol has the formula :—



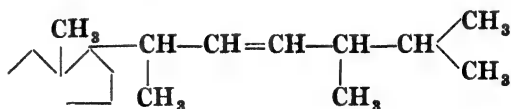
Lumisterol has exactly the same two dimensional formula as ergosterol, but is not precipitated with digitonin, presumably only differing from ergosterol in the relative position of the hydroxyl and methyl groups. It has no antirachitic activity.

In tachysterol



a profound alteration in the molecule has occurred, one of the rings having been ruptured. Tachysterol, so called because of the rapidity with which it reacts chemically, has no antirachitic action.

Calciferol



is a white crystalline substance, melting at 115°–117° C. Its maximum absorption is at 265 millimicrons. It is only stable if kept in a refrigerator in amber coloured bottles, sealed without air; dissolved in propylene glycol or maize oil it is stable, even sealed with air, at room temperatures for at least three years [252]. It loses its antirachitic properties when heated to about 180° C. Its antirachitic potency is generally said to be 40,000 I.U. per mgm., though it may be ten to fifteen per cent. higher than this [252]; esterification greatly decreases it [254].

The estimation of calciferol in an irradiated solution of ergosterol, or in any other circumstances, is very difficult. Its absorption spectrum cannot be used to give accurate estimations, as other irradiation products blur the picture; the latter also prevent any chemical estimations,

so that—apart from the use of colour reactions with antimony trichloride for the very inaccurate estimation of vitamin D_3 in fish liver oils [229, 232]—biological assay is the only means at present available. This is discussed later on p. 650.

Calciferol, if irradiation is prolonged, turns to toxisterol (substance 248) about which little is known chemically. It has an intense absorption band with its maximum at 248 millimicrons. It probably has no anti-rachitic action, though it is toxic. With further irradiation it changes to the suprasterols. It is formed in the largest amounts when alcohol is the solvent. It is chiefly of interest because a German preparation, Vigantol, was made by irradiating ergosterol in alcohol until the typical absorption band of toxisterol was at its maximum. This, of course, meant Vigantol was highly toxic and had relatively little antirachitic value.

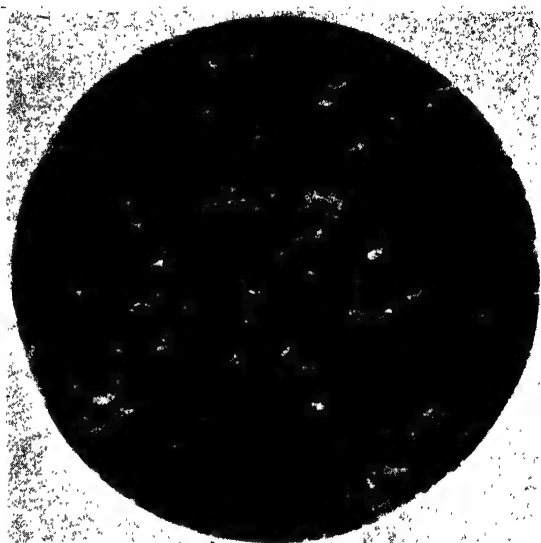
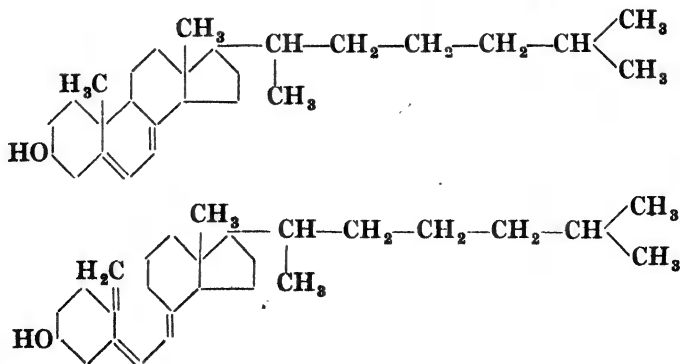


FIG. 157. Crystals of Vitamin D_3 (Calciferol).

Vitamin D_3 is formed in the same way as vitamin D_2 , only its precursor, or provitamin, is 7-dehydrocholesterol, an animal and not a vegetable sterol.

The absorption spectra both of vitamin D_3 and its provitamin are like the spectra of vitamin D_2 and its provitamin. This is the chief reason why for so long vitamin D_3 was thought to be the only vitamin D.

The formulæ for 7-dehydrocholesterol and vitamin D_3 are as follows :—



The estimation of vitamin D_3 has the same difficulties as the estimation of vitamin D_2 , and so to be accurate has to be biological (p. 650).

The stability of vitamin D₃ is very similar to that of vitamin D₂; it has been very fully investigated under various conditions and with various solvents by Huber and Barlow [252]. The effect of saponification of fish liver oils on the biological activity of the vitamin D₃ which they contain has been investigated by Bailey [254].

The antirachitic power of vitamin D₃ is the same as that of vitamin D₂ for rats, but is considerably greater for chickens [21] and probably for children (p. 655). Vitamin D₃ also has the advantage of being less toxic than vitamin D₂ (p. 647).

Vitamin D₄ is made by irradiating 22-dihydro-ergosterol. It is an artificial vitamin of no practical interest.

There are many other D vitamins, of which about ten have been more or less investigated. None as yet appear to be of more than theoretical importance. There is, however, evidence that some fish oils contain further new forms of vitamin D [23] which are especially antirachitic for the turkey [230] and are also of clinical value [209].

PHYSIOLOGY OF VITAMIN D

Research has brought us to the unusual position of knowing more about the purely artificial vitamin D₃, or calciferol, than about those forms of vitamin D which occur naturally.

Fortunately the general action of calciferol and the natural vitamins is broadly the same, so that a short account of how they differ will leave the way clear for considering the physiology they share in common.

Vitamin D₃, or calciferol, probably never occurs naturally: it was not discovered in food but artificially manufactured. Yet for some time it was considered to be the only vitamin D, and on this fallacious but obvious assumption it has been, and still is, widely used both experimentally and clinically. The confusion arose because it was found that the substance in animal fats which can be activated gave an absorption spectrum similar to ergosterol. Therefore it was argued that ergosterol was the provitamin of vitamin D, especially as ergosterol gives the strongly antirachitic substance calciferol on irradiation. In reality, however, it is 7-dehydrocholesterol, with an absorption spectrum like ergosterol, which is the substance activated in animal fats [17].

Ergosterol, it must be emphasized, is a sterol which is only found in plants, especially in the lower plants and fungi like that of ergot from which it derives its name. Animals neither make ergosterol for themselves nor absorb it from their food, though the laying hen is said to be an exception in the latter respect [24]. It is true that irradiated ergosterol, or rather calciferol, can be absorbed and utilized by animals, but this appears to be unimportant in natural diets since living plants contain no calciferol; though traces of calciferol may be formed in dead plants, such as hay and especially cacao shells, by their accidental exposure to sunlight [16].

Calciferol has the same antirachitic effect as vitamin D₃ for rats, but is far less potent for chicks [21] and is probably less potent for children (p. 655). It is also more toxic than vitamin D₃ (p. 647).

Vitamin D₃, on the other hand, is the naturally occurring vitamin. It is found in animal products such as egg-yolk, butter, and fish liver oils [75]. From any of these natural sources it seems reasonable to believe that vitamin D₃ is free of the toxic by-products of irradiation even though its origin is of course from irradiated animal sterols.

The differences therefore between vitamins D₂ and D₃ are :—

- (1) Vitamin D₂ is not a naturally occurring vitamin.
- (2) Vitamin D₂ is almost always contaminated with other possibly toxic substances and is more toxic than vitamin D₃.
- (3) Vitamins D₂ and D₃ have a different antirachitic potency for many species of animals.

The Origin of the Vitamin D of Animals. Animals gain their vitamin D in two ways : by devouring the tissues containing vitamin D of other animals, or by the direct action of the sun's rays on the provitamin in or on their own skins. Food as a source of vitamin D is generally not important ; only man in temperate and cold climates and nocturnal animals and birds are driven to use food as an alternative to sun. Vitamin D is sometimes deliberately increased in human food. This can lead to some confusion as to what vitamin is really being taken. Thus by irradiating a nursing mother or an animal vitamin D₃ is increased in their milk because their own animal sterol has been activated. The same result is obtained if the milk itself is irradiated. But if a nursing mother or animal is fed on irradiated yeast or any other form of irradiated ergosterol the increase in the antirachitic power of the milk is due to vitamin D₂ being secreted in it.

The action of the sun on the skin is profoundly interesting. The formation of the vitamin appears to occur rather on the skin than in it. Thus birds when they preen themselves remove oil on their beaks from their preen glands and spread it over their feathers, where it is exposed to the sun and activated. It is then either absorbed by the skin or scraped off the feathers by the beak and eaten. The removal of the preen gland makes birds more susceptible to rickets, and prevents ultra-violet light from having any antirachitic effect unless the feet as well as the plumage are irradiated. The fur of animals in a similar way appears to be the place where the vitamin is formed : preventing rats from licking their fur destroys the antirachitic effect of irradiation, and owls and young carnivorous birds in captivity have to be given not only the flesh but also the fur of mice or rabbits if they are to thrive [25]. All this suggests that the incessant "washing" of cats and rabbits and the apparent hunt of monkeys for each other's fleas is really a method of gaining vitamin D. In man also activation appears to occur on, rather than in, the skin. Helmer and Jansen [26] found that the fat washed off the bodies of athletes who had been exposed to irradiation before taking violent exercise was antirachitic, while fat from the skin of athletes who had not been irradiated had only a trivial potency. Irradiation of this fat made it potent. Further, the ultra-violet rays of the sun penetrate only some 0.1 mm. [27] to 1.2 mm. [28] through the skin, so that activation

must occur at least close to the surface. Possibly the old belief that too much washing makes babies fretful is due to the removal of their vitamin D leading to the fretfulness of rickets. After sunbathing it appears possible that swimming is a mistake, the activated fat being washed off the skin before it has had time to be absorbed.

The Origin of the Vitamin D of Fish. Nothing is definitely known about how fish acquire their vitamin D. The plankton in the sea which are the basis of the diet of the small fish, on which the larger fish feed, do not, according to Drummond and Gunther [29], make vitamin D, nor does it seem probable that the sun's rays activate a provitamin in the fish [30]. Thus the two ways in which animals acquire vitamins are not open to fish. One is driven back to the explanation that fish synthesize vitamin D, though why they do so, or what advantage it is to them to store so much is obscure. Apparently the vitamin in many cases is not only vitamin D₃ [75] but also one or more new vitamins of an even higher antirachitic value [28, 209, 280].

Assimilation of Vitamin D. In birds and furry animals we have already seen that the vitamin having been formed on the feathers and fur is, at least in part, swallowed during preening and licking. In man, whether the vitamin is made in or on the skin, it must be absorbed from the skin because irradiation will cure rickets. This, and the fact that vitamin D is active when injected, proves that no essential change has to take place in it during digestion before it can be used by the body.

Bile is essential for the absorption of vitamin D. Taylor and his co-workers [31] found that dogs with biliary fistulæ did not absorb vitamin D when given by mouth unless bile salts were given at the same time. Other investigators, among whom is Heymans [31], have confirmed these results by very similar experiments on rats or dogs. No analogous work has been done on man, but there seems no reason to doubt that similar results would be obtained.

Liquid paraffin, or mineral oil, hinders the absorption of vitamin D, probably because the vitamin is dissolved in the oil and so is excreted with it in the fæces. Smith and Spector [32] reported that rats on a non-rachitic diet develop rickets if as much liquid paraffin is added to their food as would correspond to the amount taken by man for constipation. Five times the normal requirements of cod liver oil had to be taken by the rats to counterbalance the effect of this amount of liquid paraffin. The same workers also found that the antirachitic value of irradiation is reduced by liquid paraffin, since when it is included in the diet of rats they require a larger amount of irradiation to avoid rickets. This, it is suggested, proves that vitamin D, formed by irradiation, has to be excreted into the gut to function. A more probable explanation is that liquid paraffin also hinders the absorption of calcium and phosphorus, so making the diet more rachitic (p. 640). Though nothing is known of the effect of liquid paraffin on the absorption of vitamin D in man, it would seem that the use of it and its emulsions as aperients should be avoided, especially as it also interferes with the absorption of vitamins A, E and K, and also damages the liver [285]. The advice given to patients to take liquid paraffin several times a day after meals therefore is bad.

Storage of Vitamin D. The various tissues of the body store vitamin D for varying periods. The following times in weeks are given by Heymann [33]: brain, 1-2; red blood cells, 5-6; small intestine, 5-8; large intestine, 6-8; lung, 6-9; kidney, 6-9; liver, 8-12; blood plasma, 8-12 or longer. Other reports mention shorter times, especially for the liver and the lungs; in the latter an enzyme was even thought to be present which destroyed vitamin D, but its disappearance was probably due to not keeping the lungs frozen until the estimation was made. There are only a few estimations of the amounts of vitamin D in the various tissues of the body because lengthy biological methods have to be employed (p. 650). Warkany and Mabon [160] report that the normal level in the blood both of adults and children varies from 66 to 165 I.U. per 100 c.c., with seasonal variations. When adults take from 50,000 to 500,000 I.U. of vitamin D daily the level in the blood rises to between 9,000 and 18,000 I.U. per 100 c.c. [195]. Vollmer [196] gave three children dying from incurable diseases massive doses of vitamin D shortly before their death. After death no vitamin D was found in the tissues of one child; the second child had large stores in the brain, liver and skin; the third child stores in the skin, thyroid and parathyroids, but none in any part of the brain, liver, thymus, or blood. The explanation of these confusing observations is not clear.

Excretion and Destruction of Vitamin D. The depletion of the stores of vitamin D in the body are caused partly by its excretion into the gut and partly by its destruction in the body, though there is no knowledge about how or where the latter takes place. The former is said by Heymans [81] to occur in the upper third of the small intestine. Lawrie and others [184] state that no vitamin D is normally excreted in human or canine urine, but one patient with osteomyelitis and nephrosis excreted 20 I.U. daily when taking 9,000 I.U. by mouth, which is reminiscent of the urinary excretion of vitamin A (p. 20).

Rachitic Diets, apart from Lack of Vitamin D. The composition of the diet, apart from its vitamin D content, has a great effect in rickets. Thus it is necessary to consider briefly what backgrounds of diet may complicate investigations on vitamin D.

Calcium and Phosphorus. Not only the absolute amounts of these elements, but their proportions to each other are important. In rats, in spite of a deficiency of vitamin D, rickets does not occur unless the calcium and phosphorus intake is unbalanced.

In man and dogs rickets occurs on normal diets if vitamin D alone is deficient, but even here the calcium and phosphorus of the diet is a deciding factor in the amount of vitamin D which will prevent rickets. As they become more unbalanced in the diet, so does the amount of vitamin D required increase.

The usual ricket-producing diets contain an excess of calcium and act by forming insoluble phosphates in the bowel, which cannot be absorbed. Diets deficient in calcium or phosphorus really have the same ultimate effect—the inadequate supply of minerals for calcification.

Metals which form Insoluble Phosphates. Any metals, such as magnesium [85], lead [86], strontium [87], thallium [88], and beryllium [89],

which like calcium form insoluble phosphates, will accentuate rickets in the same conditions as will extra calcium.

Fluorine. Fluorine increases the deposition of calcium in the bones of rachitic rats and delays their death, though it hinders the antirachitic action of vitamin D [182]. It also protects the teeth against decay (p. 689).

The "Acidity" of the Diet. Foods with an alkaline ash tend to decrease the absorption of phosphorus and calcium from the gut, but to increase their retention in the body, while foods with an acid ash have the opposite effect.

Organic Acids. Citric and tartaric acids and their salts may have a beneficial effect on rickets. This has nothing to do with altering the acidity of the bowel. Day [40] found that citrates only have an effect when there is phytic acid in the diet and a high calcium phosphorus ratio. He suggests this is due to the citrate forming a complex with the calcium, so enabling the phosphorus of the phytic acid to be absorbed instead of forming an unabsorbable compound itself with the calcium (see below).

Phytic Acid and Cereals. The bad effect of cereals, especially oatmeal, on rickets, has been known since Trousseau's day (1865), though this is probably not true for Scotch oatmeal [215]. Harrison and Mellanby [41] have shown the action of cereals is due to their phytic acid combining with the calcium of the diet to form a compound which cannot be absorbed. The effect therefore of increasing the cereals in the diet is the same as decreasing the calcium. The phosphorus, of course, of the phytic acid is also lost to the animal when it combines with calcium.

The subject suddenly attracted considerable attention when the Government came in April, 1942, to the tardy decision that it was necessary to substitute eighty-five per cent. extraction flour for the seventy per cent. extraction "white" flour which hitherto had been used for most of the bread and pastry in England. It is regrettable that this decision was made to save shipping space and not to improve the diet of the nation. Among the many protests of millers, politicians and illiterate gastric hypochondriacs against this dietetically wise legislation was the possibly valid objection that the increased phytic acid in eighty-five per cent. extraction flour would dangerously decrease the absorption of calcium, especially in view of the dearth of foods rich in calcium during war, such as milk and cheese. To compensate for this effect of phytic acid it was proposed that calcium should be compulsorily added to the flour. *The Times* and also Parliament and the medical press, throughout the winter and spring of 1942 and 1943, hotly argued about the proposal with common sense, bias, ignorance and science. One of these carried the day and 7 ounces of calcium carbonate was added to every 280 pounds of flour. For an excellent summary of and references to the spate of speeches and correspondence on this subject and also on the quaint earlier idea of the Government to add synthetic vitamin B₁ to white flour the reader should consult the fascinating review by Lepkovsky [212] on "The Bread Problem in War and in Peace." It is to be hoped that the actions of the Government as shown in this review will appear vacillating rather than venial. The outstanding papers on the effect of high extraction flour on mineral absorption in man are those by McCance and Widdowson [218], which

strongly support the addition of calcium to such flour. The work, however, of Krebs and Mellanby [214] and of Cruikshank and others [215], suggests that this addition may be unnecessary and the work of Yudkin [216] shows that calcium phosphate might be a better salt to add than calcium carbonate, especially for young children [216, 217], and nursing mothers [218]. It would also seem possible that the efficiency of calcium absorption is largely governed by the needs of the body [219] so that investigations which show a negative balance on diets largely composed of high extraction flour may be invalid because the experimental subjects never lost sufficient calcium to bring about an increase in the efficiency of its absorption. This endogenous factor for the control of calcium absorption appears, in animals at least [219], to be dependent on vitamin D and to be only present during growth.

Fat and Carbohydrate. The amount of fat in the diet is important. McDougall [161] counteracted the effect of cereals by a high fat intake, attributing this to the formation of calcium soaps which were rendered soluble by the action of bile, and so were easily absorbed. She later thought the cholagogue action of fat was also involved. Knudson and Floody [161] report that there is an optimum intake of fat for the prevention of rickets, larger or smaller amounts than this having a less beneficial effect, which may be due to the anticacifying action of fat being reversed when the phosphorus of the diet is increased from low levels to high [221].

Outhouse and others [185] have shown that lactose, but not sucrose or starch, increase calcification and the retention of calcium, magnesium and phosphorus. This observation may be explained by the work of Laszt and others [250] who showed that in rachitic animals the phosphorus which is excreted into the gut for the absorption of glucose is poorly reabsorbed. This means that any sugars which cause the gut to excrete phosphorus will tend to be rachitogenic because the excreted phosphorus, if there is not sufficient vitamin D for its reabsorption, will be precipitated by calcium and magnesium and so all three will be lost to the body. Sugars like lactose which do not cause the secretion of phosphorus will, by contrast to sugars like glucose, appear to be antirachitic.

Effect of Vitamin D on Calcification : Rickets. Vitamin D regulates the metabolism of calcium and phosphorus (p. 648).

Since, by definition, vitamin D cures or prevents rickets, most physiological investigations have been on its effect on bone metabolism and the elements which chiefly form bone, calcium and phosphorus. It must, however, be admitted that the action of vitamin D is still very obscure. This obscurity is partly due to the complexity of the problem, partly due to the fact that too much attention has been paid to the bone changes in rickets and not enough to the wider metabolic disturbances. It should also be remembered that in spite of Mellanby using puppies for his original work, most later investigations have been carried out on rats. Rats, unlike puppies, are not good animals for this work as on a normal diet they are immune to rickets. They are only susceptible when their calcium and phosphorus intake is grossly unbalanced, which means that when rickets is produced it is not due to a pure vitamin D deficiency, but to a deficiency

grafted on to an already abnormal animal. The number of ways growing bone can develop abnormally is limited, so that rickets in rats is the "final common path" for a number of different metabolic disturbances of vitamin D, phosphorus or calcium, or of these combined together (p. 689). With these reservations as to the true meaning of much of the work done we may consider the importance of vitamin D for bone metabolism.

During growth the long bones of the body increase in length by the calcification of the cartilage between the diaphysis and the epiphysis.

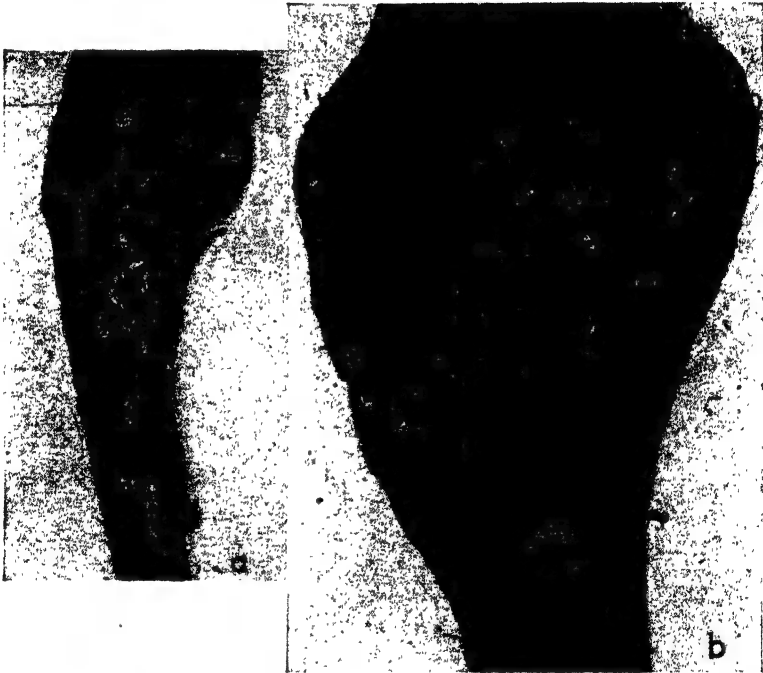


FIG. 158. Photomicrographs of costochondral junctions of a normal and a rachitic dog. The diet of both dogs contained a large amount of oatmeal, but the normal dog (*a*) received cod liver oil while the rachitic dog (*b*) received olive oil. Note in (*b*) the swollen or "beaded" junction, and the uneven line of calcification.

This cartilage on its epiphyseal side is continuously growing, while on its diaphyseal side it degenerates, being then invaded by capillaries, and osteoblasts or bone forming cells. Bone salts are then laid down in the degenerating cartilage, but the formation of new cartilage keeps pace with its degeneration, so that growth in length occurs by the invading bone on the diaphyseal side of the cartilage always chasing the retreating new cartilage on the epiphyseal side. Growth ceases when no more cartilage is formed, so that the diaphyseal bone reaches and fuses with the epiphysis. The thickness of the bone is increased by osteoblastic activity underneath the periosteum.

In rickets the band of cartilage widens because it goes on growing but

ceases to degenerate. A large number of osteoblasts and capillaries appear between it and the diaphysis forming a mass of osteoid tissue, that is "the organic part of bone without the inorganic." No calcification takes place, since degeneration has ceased in the cartilage. The large amount of osteoid tissue and expanded cartilage causes the classical swellings, for instance, of beaded ribs and enlarged ankles and wrists. Under the periosteum of the bone calcification stops though there is an increase in vascularity and osteoblasts forming osteoid tissue. The finer structure of the bone and the trabeculae of the marrow cavity is affected, because decalcification also occurs. Clinically this results in weakening of the bones with bending and fractures.

The X-ray picture in severe rickets shows these changes. In normal growing bone the end of the diaphysis is smooth and there is a clear space between it and the epiphysis. But in rickets the end of the diaphysis is uneven and ragged because the degeneration of the cartilage and subsequent calcification has stopped unevenly. A faint curved shadow along the edge of the cartilage may sometimes be seen extending towards the epiphysis: this is the faintly calcified perichondrium round the swelling caused by the uncalcified mass of osteoid tissue and overgrown cartilage. The shaft of the bone also shows rarefaction with a general coarsening of the structure, while the outline is irregular and blurred by the shadowless



FIG. 159. Fetal rickets in a Chinese baby. A section of the distal end of the ulna stained with silver nitrate. Note the expanded cartilage and osteoid tissue, and the ragged diaphysial end of the ulna. (See also Figs. 164 and 166.)

osteoid tissue under the periosteum (*see* X-rays in Figs. 166, 170 and 172.

When healing starts under the influence of vitamin D the first change (which can be shown histologically within twenty-four hours) is the resumption of degeneration of the cartilage cells nearest to the diaphysis. This is rapidly followed by capillary penetration and the laying down of bone salts. The result is that a line of preparatory calcification is formed close to the ragged edge of the diaphysis which can be seen on X-ray examination, forming the "line test" for healing rickets. Bone salts are

also laid down in the osteoid tissue at the ends of the bones and along the shafts, so that in both places denser shadows appear in an X-ray (Fig. 160).

In adult animals deprived of vitamin D the picture must be modified because growth is no longer taking place. The changes have to be limited to the decalcification of the bones, and the formation of osteoid tissue under the periosteum, with a resulting weakness giving clinically the

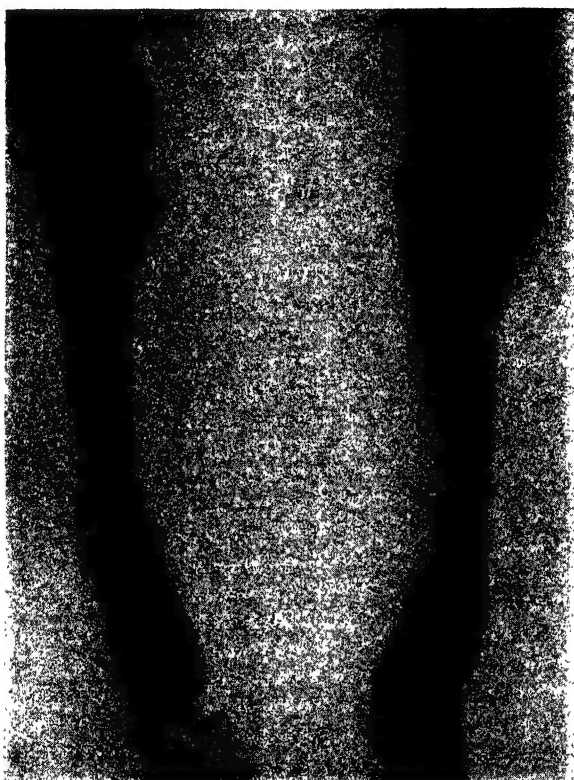


FIG. 160. Healing rickets with fractures in an English child. Note the lines of dense calcification at the ends of the diaphyses. Bands of newly formed bone can be seen at each end of the shafts, distinguished from the bone formed before therapy was started by their fine and homogeneous structure. Dense new periosteal calcification along the shafts is also visible.

picture of osteoporosis or, in severe deficiencies, osteomalacia. The rôle of vitamin D in the calcification of the teeth is discussed on p. 689.

The other changes which occur in rickets are :—

(a) The amounts of calcium and inorganic phosphorus in the serum are altered. In experimental rickets these elements largely mirror their content in the food, so that rickets can be produced with widely varying amounts and proportions of these two present in the serum. In human rickets the phosphorus is generally low, the calcium normal, but the latter

may be grossly reduced. In osteomalacia the calcium is often reduced to tetanic levels, but it may remain nearly normal with very low phosphorus levels.

(b) The phosphatase of the blood is constantly raised in rickets and, to a less degree, in osteomalacia. This rise, however, can be prevented in animals by depleting the diet of manganese [199].

(c) The calcium and phosphorus of the faeces is increased, of the urine decreased.

(d) The skeletal muscles lose their tone, and the ligaments become lax.

(e) The smooth muscle of the gut also loses its tone. This is important, as Yoder [84] reports that it may increase the time food takes to pass along the gut by as much as twenty-six per cent. He points out that this relative stagnation of the food means that chemical changes take place in the gut as the result of decomposition of the food, and not as the direct result of lack of vitamin D. He believes the alkalinity of the faeces in rickets is due to this decomposition, since the reaction can be restored to normal either by vitamin D or purgatives.

The Relation of Vitamin D to Growth. Stimulation of growth, as apart from the prevention of rickets, is an important function of vitamin D. Such clinical observers as Gardiner-Hill [55] and Still [56] state that a deficiency of vitamin D decreases growth quite apart from loss of stature due to rachitic deformities (Figs. 156, 165), and this has been confirmed in rats [220]. Large scale investigations on children also show that amounts of vitamin D above those necessary to prevent rickets lead to increased growth [57]. Norman's important work [58] on the difference in height between children of the richer and poorer classes also would suggest that vitamin D is related to growth, since it is one of the most deficient vitamins in the diet of the poor; Harris [162] in 1933 stated that eighty to ninety per cent. of the elementary school children in London gave evidence of having had some degree of rickets.

Whether it is an advantage to the individual to be taller is debatable. That children and experimental animals grow more on large amounts of vitamin D does not mean that such doses of vitamin D are the correct ones. It may equally well be held that large amounts of vitamin D stimulate excessive growth. Jeans and Stearns [59] found that when vitamin D is given in still larger doses growth is impaired, even though toxic symptoms are absent. Analogous results are reported by Speidel and Stearns [163], since giving infants 300 to 400 I.U. of vitamin D daily caused an earlier eruption of the first deciduous incisors than did larger or smaller doses. The body appears to have no power to regulate the amount of vitamin D it absorbs in the food; whether it can regulate that formed in the skin by irradiation is unknown. But as the latter appears to be the natural way of acquiring vitamin D experiments suggesting the value of a high consumption of the vitamin should only be accepted if the results are the same as are given by irradiation. Any results which appear to suggest feeding is better than irradiation may mean that they are not truly better, but are the effect of abnormal stimulation. If growth is taken as the criterion for measuring the correct dose of vitamin D we should know what is optimum growth. We do not.

The Relation of Vitamin D to the Endocrine Glands. *The Parathyroids* [60]. The parathyroids being so intimately connected with the metabolism of calcium and phosphorus must have some direct or indirect relationship to vitamin D. It has even been suggested that vitamin D only acts through stimulating these glands. This cannot be so, since the action of vitamin D and parathormone is quite different. With small doses the former keeps the serum calcium and inorganic phosphorus normal, and causes a positive balance of both in the body. The latter raises the blood calcium, after decreasing the inorganic phosphorus by diuresis, and causes a negative balance of both. Excessive amounts of the hormone and vitamin have different actions on bone: the former causes decalcification and replacement with fibrous tissue and giant cells, while the latter causes dissolution of the trabeculae with no fibrous replacement.

Clinically the difference is important, since rickets is cured by vitamin D but made worse by parathormone. Further, in parathyroid tetany the beneficial effect of parathormone wears off, but the effect of vitamin D does not [60, 286].

In rickets and osteomalacia the parathyroids often appear to be enlarged and overactive: vitamin D causes them to revert to a resting state [145].

Possibly vitamin D should be considered as regulating the metabolism of phosphorus and the parathyroids as regulating phosphorus excretion. Sometimes the hormone acts with and sometimes against vitamin D according to the needs of the body.

The Thyroid and Pituitary. The basal metabolic rate is decreased in rickets, but this is explained by Nicolaysen [61] as being due to decreased activity, since he found in narcotized rats that the carbon dioxide output was the same whether rickets was present or absent.

Vitamin D in doses on the threshold of toxicity raises the basal metabolic rate by stimulating the thyroid *via* the thyrotrophic mechanism of the pituitary [61, 63, 64]; it seems doubtful if this can have any clinical importance. With toxic doses the thyroid appears to be overactive and there is an increase in the eosinophils of the pituitary [65]. Vitamin D, and calcium chloride, though not calcium carbonate, increase the size but not the iodine content of the thyroid glands of rats on a slightly goitrogenic diet [287].

The Thymus. There is some evidence that the removal of the thymus in animals increases the severity of rickets and decreases the effect of vitamin D [66, 67].

The Sex Glands. In osteomalacia the regularity of the menses, and the disastrous recurrent pregnancies suggests that lack of vitamin D has no effect on reproduction in woman. It has been reported by Reed and his collaborators [45] that huge doses of vitamin D have occasionally increased sexual capacity and libido (even to an inconvenient extent) and also increased the regularity of the menses. Toxic doses decrease spermatogenesis in the dog [65] but in the rat have no effect on this or ovulation [68], though lack of vitamin D in the latter animal causes dioestrus [288]. In mice the endosteal bone formation caused by oestrogens is not dependent on an adequate supply of vitamin D [289].

The Relation of Vitamin D to the Blood. Gray and Ivy [178], and McNealy and others [179] have shown that vitamin D prevents the hæmorrhagic tendency which is so common and dangerous in patients with jaundice and hepatic insufficiency (p. 693). Why the vitamin has this effect is obscure: it is not through any alteration in the blood calcium [178] or through correcting a prothrombin deficiency [200]. Vitamin D in man appears to have little or no effect on the formation of erythrocytes or leucocytes, or on the coagulation time or sedimentation rate [45]. In dogs large doses decrease capillary permeability [256]. The thrombocyte count in rats is said to be increased by large doses of vitamin D [180] and rachitic rats absorb iron and form hæmoglobin slightly less well than animals receiving vitamin D, especially when this is from fish liver oils [251].

The Relation of Vitamin D to Infection. During the last century in England cod liver oil was greatly prized for its value in tuberculosis [74]. This, added to the commonness of catarrhal infections in rickets, and the clinical value of sunshine and ultra-violet light in improving the general condition of consumptive and convalescent patients, has led to a widely held belief that vitamin D increases the resistance of the body to infections. But the results of animal experiments in which the formation of antibodies, etc., have been studied are indecisive and conflicting. Most probably the only value of vitamin D is the indirect one of avoiding the debilitating effect of a deficiency. It must be remembered that when cod liver oil or ultra-violet light are used clinically the oil and vitamin A of the former and the general stimulation from the latter are valuable quite apart from any action of vitamin D itself. Reed and his collaborators [45] in 1939 summed up their excellent review of the literature on vitamin D and infections by saying ". . . there is no proof of a specific effect in any type of infection with the possible exception of tuberculosis." More recently Tomey [246], using monkeys, has shown that rickets reduces resistance to the virus of poliomyelitis when this is injected into the wall or lumen of the gut or into the suprarenals and, further, that rickets reduces the effectiveness of vaccines. Rachitic nerves ground up with the virus absorb it while normal nerves do not. Weaver and others [247], however, having inoculated cotton rats with the virus in every possible manner could not confirm that lack of vitamin D had any effect on susceptibility or on the development of resistance. Tomey's findings are probably of no clinical importance since the seasonal incidence of poliomyelitis is not that of rickets, nor has poliomyelitis ever been found to select the rachitic and poorly fed. The clinical results of treating infections with vitamin D are discussed on p. 695.

Effects of Excessive Vitamin D. All forms and preparations of natural and synthetic vitamin D are toxic when given in sufficiently large amounts, though the belief still lingers that pure vitamin D itself is not toxic because the first workers with massive doses of the vitamin ascribed its toxicity wholly to the toxic impurities, such as toxisterol, which were present in the very impure irradiated ergosterol which they used. Actually pure or highly purified preparations of vitamin D₂ and concentrated fish liver oils are toxic for the rat [22] and the dog [43, 45, 231] and man (p. 696). Vitamin D₂ is definitely more toxic than vitamin D₃ for rats [22] and

dogs [231] and so it is reasonable to suppose it would be so for man, though no work has been done on this subject. Toxicity is reduced in animals by very large amounts of vitamin A [22, 187, 231]; this has not been confirmed in clinical work [45], though vitamin B₁ or yeast are often stated to be of value [45, 233].

Excessive doses of vitamin D mobilize the phosphorus and calcium from the tissues of the body, thus broadly having an opposite effect to normal doses. The soft tissues tend to become calcified, the bones to be rarefied—in growing bone the cartilage acts as a soft tissue. The soft tissues most affected are the tubules of the kidneys [48] the media of the arterioles of the kidney [46] and the media of the large blood vessels, though the bronchi, heart and stomach are also involved. The aorta, for instance, in animals kept for some time on sublethal doses looks exactly like that found in old atheromatous men. This metastatic calcification takes place after the tissues have already been damaged by the excess of vitamin D; it is thus a secondary change, and not the primary one [48]. Casts composed of calcium salts are often present in the urine if this is not too acid. If the toxic doses of vitamin D are stopped the calcareous deposits may largely disappear [44]. The serum phosphorus and calcium tend to be grossly raised but not always—so that a raised blood calcium does not of necessity give a warning that the amount of vitamin being taken is toxic [45]. A diet rich in bone salts increases the metastatic calcification, but a diet deficient in salts does not decrease the fundamental damage to the tissues. Oppel [193] states that metastatic calcification is increased in rats when their renal function is impaired either by a diet deficient in vitamin A (p. 37) or by partial nephrectomy. In man [45] the blood pressure is not affected, but in dogs mild toxicity causes hypertension and severe toxicity hypotension [45], while in rats hypotension occurs with mild toxicity and severe toxicity has no effect [240]. With severe vitamin D poisoning animals develop severe diarrhoea and loss of weight and may die in a few days. The clinical picture and post-mortem findings in human cases poisoned by vitamin D are described on p. 696.

Fundamental Nature of the Action of Vitamin D. The effects of vitamin D are best explained, though it must be admitted not completely, by considering that its action is primarily on phosphorus metabolism throughout the whole body [76]. Thus vitamin D not only mobilizes phosphorus from the tissues, so aiding its combination with calcium [50] by converting organic phosphorus into an inorganic form [47], but it also has an effect on the metabolism of phosphorus during muscular work [51].

Nicolaysen has shown, by most careful work on rats, that calcium absorption is increased by vitamin D [52], but that this effect is largely dependent on the needs of the body for calcium, especially in the young [219]: in the adult both vitamin D and the needs of the body for calcium have much less effect on absorption, which has been confirmed for women [253]. Nicolaysen [52] did not find that the absorption of phosphorus from isolated loops of the intestine was different in rachitic and normal animals, though Laszt [250], using the same technique, reports that the

absorption of phosphorus is decreased in rickets. But whether or no vitamin D directly affects phosphorus absorption it is probable that its effect on calcium absorption is really a secondary effect due to it rendering the phosphorus in the cells of the gut wall sufficiently labile for the calcium to combine with it and so be absorbed [77].

Accepting this view of the action of vitamin D, calcification is started by the conversion of the organic phosphorus of the serum and bone to inorganic phosphorus. This so raises the concentration of the latter that it can combine with the calcium of the serum to form insoluble calcium phosphate, aided in the neighbourhood of growing bone by the action of Robison's bone phosphatase on hexose phosphoric esters [58].

It must be stressed that bone salts are not laid down because an increase in inorganic phosphorus in the serum automatically causes precipitation of calcium phosphate: they can only be laid down by the action of living cells [48] when the concentrations of salts bathing these are sufficiently high.

Rachitic bone and cartilage have no fundamental inability to lay down bone salts, since they do so if they are placed in normal serum or suitable salt solutions [48]. In living rachitic animals the injection of inorganic phosphorus and calcium also causes calcification [49], since the salts are artificially put into the fluid bathing the cells in a form which is only produced naturally by the action of vitamin D.

The poor tone of the skeletal and visceral muscles in rickets is possibly due to the effect vitamin D has on phosphorus metabolism in muscular work [51].

The damage done by excessive amounts of vitamin D (p. 647) may be due to an excessive mobilization and conversion of organic phosphorus to inorganic phosphorus not only in the soft tissues but also in the bones: a mobilization which is injurious in itself, only secondarily leading to metastatic calcification [48] when enough calcium is present to combine with the liberated phosphorus.

Another theory of the toxic action of vitamin D is that it is due to an impurity formed during irradiation, such as toxisterol (p. 685). In favour of this is that unit for unit the least purified products of irradiated ergosterol are the most toxic [54]. Toxic symptoms again often occur only after some intestinal upset which might have led to decomposition of vitamin D in the gut before absorption with the production of injurious substances [45]. Vitamin B₁ has a protective action against overdosage of vitamin D, which may be explained by the value of the former for the proper functioning of the gut, which would decrease any tendency to intestinal putrefaction [45]. As, however, very pure preparations of calciferol have a toxic action when injected one must also postulate that the cells of the body cannot completely destroy huge doses of vitamin D, but form from it some toxic substance like toxisterol. Here again adequate vitamin B₁ should aid the cells in the complete destruction of vitamin D. This theory does not run counter to the theory that excessive vitamin D upsets phosphorous metabolism, but only tries to explain further the underlying mechanism. From the clinical point of view it is an added argument against the use of irradiated preparations.

UNITS OF VITAMIN D

The international unit of vitamin D is the amount of vitamin D equal in antirachitic potency to 1 mg. of the international standard solution, which is equal to 0.025 micrograms of crystalline vitamin D₂ (calciferol). This international unit is the same as the United States Pharmacopœia unit. Both the English and American pharmacopœias give details of how substances are to be compared for their vitamin D content against the international standard. Rats must be the animals used for assay, so all international units are also "rat" units.

The A.O.A.C. unit or "chick" unit was introduced by the American Association of Official Agricultural Chemists. It has the same value as the international unit, but chicks, not rats, are the animals used for assay (p. 651). This means, for all practical purposes, that A.O.A.C. units are international units of vitamin D₃ since vitamin D₂ has little antirachitic value for the chick, though both vitamins have the same value for the rat [21].

ESTIMATION OF VITAMIN D

Biological methods have to be used for accurately estimating vitamin D because chemical or spectroscopic methods are only of value for the very crude assay of fish liver oils (p. 634). For full details of the biological methods Coward's book [164] should be consulted. Four methods are commonly used.

The "Line Test." This is based on the cure of rickets. Two identical groups of rats are fed on a rachitic diet until rickets has developed. To the diet of one group is then added a standard preparation of vitamin D of known potency, and to that of the other group the substance to be assayed. This curative period requires about ten days. The rats are then killed and the distal ends of the radius and ulna in each rat are examined for calcification by splitting the bones and putting them in a solution of silver nitrate. The bone which has been deposited during the curative period is thus stained, showing as a black line. By comparing the "lines" of the two groups of animals the potency of the material being assayed can be deduced. Instead of staining with silver nitrate after death sodium alizarinate may be injected into the animals during life. This stains newly deposited bone, thus making it easy to see [166]. The incisor teeth of rats, according to Irving [255], show a more rapid and more delicate response to vitamin D than do the epiphyses.

In every fresh assay the standard vitamin D preparation must be used as a control, since the response of a colony of rats to the same amount of vitamin D varies greatly at different times. The number of rats used depends on the material being investigated. If the potency of this is not already roughly known thirty-six animals will be required, so that the standard and the substance being tested may be compared at three different levels of intake. Where large numbers of estimations are being performed the construction of a "curve of response" as in vitamin A estimations saves time.

The X-ray Method. This is similar to the line test, but the degree of calcification is judged radiographically.

The Bone Ash Method. This method depends on the prevention of rickets. Groups of rats are fed for from four to six weeks with varying levels of the standard preparation and that being tested. They are then

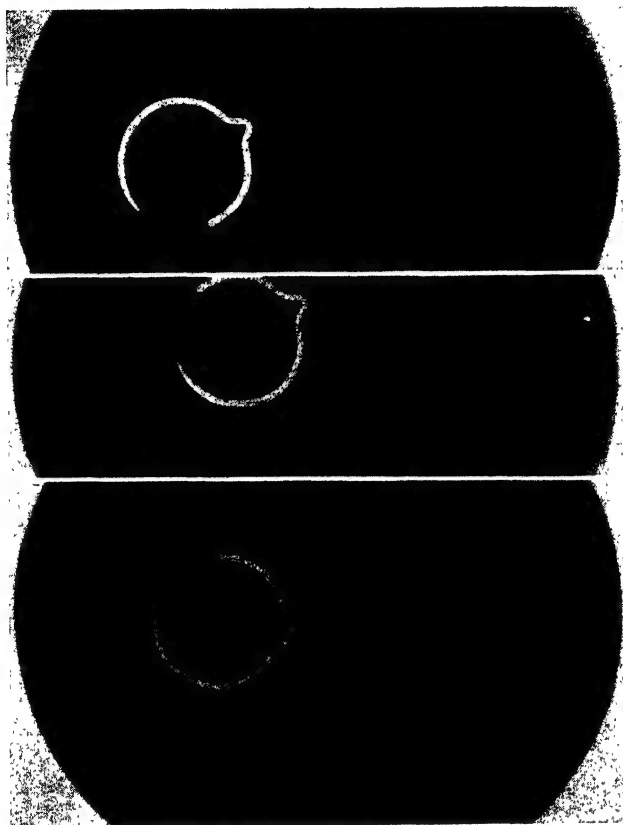


FIG. 161. X-rays of the tarso-metatarsal joints of five weeks old chicks used in Olsson's radiographic method of assay of vitamin D. The upper X-ray is from a chick receiving natural vitamin D₃ from cod liver oil, the middle X-ray from a chick receiving the same number of International Units of synthetic vitamin D₃ (calciferol), and the lower X-ray is from a chick receiving no vitamin D. Note in the top X-ray the greater calcification and the narrower tarso-metatarsal gap. (See also Fig. 162.)

killed and the bones of the femora ashed, using the metaphyses alone being most satisfactory [222]. Comparison of the weights of the ash from the various groups of animals shows how much calcification had occurred. This method is more arduous than the others but gives more accurate results.

Olsson's Radiographic Technique. Here chicks are used instead of rats, and the antirachitic potency is gauged by radiographic measurement

of the tarso-metatarsal gap (Fig. 161). Baker and Wright [165] state that this method is as accurate as the bone ash method for chicks, and has the great advantages that records are easy to keep and that the birds are not sacrificed. Since, however, the biological activity of vitamin D_2 is less than that of vitamin D_3 for chicks, it is necessary to give the control birds a reference oil containing vitamin D_3 and not vitamin D_2 , when substances containing vitamin D_3 are being assayed.

The results obtained by these methods are accurate to within \pm ten per cent. It is important that the animals should eat all their diet, especially the phosphorus and calcium, and fat. When any of these are present in the substance being tested equivalent amounts must be added to the diets of the control animals.

SOURCES OF VITAMIN D AVAILABLE TO MAN

Sunlight and Ultra-violet Light.—Sunlight acting directly on the body should be the way in which vitamin D is obtained. But in northern climates and large cities clothes, window glass, smoke, and clouds cut off most of the active rays. Even so by letting infants and children be exposed as far as possible out of doors and by opening nursery windows much benefit is gained. In one hospital in the heart of London it is found that infants always thrive better if they are out on a roofed verandah whatever the weather. This raises the point that the action of sunlight and fresh air is not only due to the formation of vitamin D but also to a general tonic effect on the body; the fluctuating temperatures on the skin, for instance, cause a stimulation of the thyroid-suprarenal mechanism.

Sunlight in excess is a powerful, though delayed, poison which may cause not only severe sunburn, but also fever, headaches, general malaise and shock. As these symptoms do not appear for some hours after sun-bathing no warning is given at the time that it has been too prolonged. It should therefore be used with care, especially for infants. The eyes and head must be shaded by a wide-brimmed hat. As some children are more sensitive than others a ten-minute exposure of the arms and legs is enough for the first few days. The duration and amount of the body exposed may be gradually increased, but complete exposure of infants for more than half an hour twice a day is excessive. When the sunlight is very intense sun-bathing is best in the cooler parts of the day—early morning and evening. With adequate sunlight the amount of exposure recommended above will cure rickets as rapidly as the doses of vitamin D commonly used. For adults sun-bathing is of value, but the prolonged sudden exposure of city people on short holidays is harmful.

Artificial sunlight, given either by the carbon arc or mercury vapour quartz lamp, is an excellent substitute for sunlight, having the same value in the prevention and cure of rickets and also the same tonic effect. Hill and Laurie [27] found in a carefully controlled experiment that children benefited more from irradiation than cod liver oil, their weight, appetite and sleep being improved, the number of colds decreased and nervousness lessened. But irradiation needs to be used with caution. Both the duration of exposure and the distance of the bather from the lamp must

be carefully supervised. It is essential that the eyes are always protected with coloured glasses. Both adults and children may be unduly sensitive to irradiation, especially those who are fair. The first exposure should be of short duration to make certain there is no intolerance. A short transitory erythema should be produced, but any other symptoms suggest the exposure is too prolonged. We have seen twenty-five people who had to go to bed with severe sunburn because of over exposure at the christening party of a new lamp. Since the various lamps now sold vary greatly in the intensity of the ultra-violet rays they emit detailed instructions for their use cannot be given here, but must be obtained from a therapist or the makers.

Food. The dietetic sources of vitamin D can be divided into (a) normal foods, (b) fortified foods, and (c) concentrated natural and artificial preparations.

Normal Foods. Vitamin D is poorly represented in food. The only good sources are dairy produce, fish, and dripping. Green vegetables, in spite of all belief to the contrary, do not contain vitamin D.

The value of all dairy produce depends on the diet and exposure to sunlight of the hens and cows. Hens' eggs contain nearly three times as much vitamin D in the summer as in the winter. The eggs of commerce produced in abnormal numbers by birds in coops may be almost valueless from the point of view of vitamins D and A.

English butter may only contain 3 I.U. of vitamin D in 1 ounce in the winter, though in the summer there may be 180 I.U. These figures are extreme values: butter from cows which are kept outdoors in the winter should never have such a low winter value. The importance of butter is shown by Friend's [150] report on the health of the boys at Christ's Hospital School. In 1918 the War made it necessary to give the boys less milk and unvitaminized margarine instead of butter. The average fracture rate till then had been 0.75 per cent. During the next four years the average fracture rate rose to 1.78 per cent., in 1922 even being as high as 2.88 per cent. Yet in 1922 all the War rationing had disappeared, the diet being as good as it was before 1918. The only difference was that margarine was still being eaten instead of butter. When butter was again given the fracture rate sank to normal. The explanation offered is that the vitamin D of the butter saved the boys from mild rickets or osteoporosis. But the amount of vitamin D in butter is small, so that it seems possible that the fat of the butter itself or some as yet unidentified factor in butter (p. 792) or the form in which it contains vitamin D was the important factor. In favour of this is the observation by Boer [72] that whole butter has eight times the antirachitic value of its unsaponifiable fraction, which suggests that butter, like milk, increases the potency of the vitamin D it contains.

Milk is an extremely valuable source of vitamin D. This is not due to the actual amount of vitamin D present, which is relatively small, but because its antirachitic value is greatly enhanced when it is given in milk. Thus Hess and Lewis [18, 71] reported that unit for unit "yeast" milk (p. 655) was five to ten times as antirachitic as calciferol dissolved in oil. Similar observations have been made by Drake [19], May and Wygant [19], and many others. Supplee and his collaborators [70] have shown that the

increased antirachitic effect of vitamin D when given in milk is due neither to better absorption of diluter concentrations nor to finer dispersion, but is the result of vitamin D forming a compound with the lactalbumin of the milk. This compound is more effective than vitamin D alone. It must also be remembered that the lactose of milk is of value in aiding calcification; cane sugar and starch have not this property [185]. Human is milk discussed on p. 660.

Sir John Russell [198] has pointed out that milk at present need not conform to any standards as regards its vitamin content. Legislation should be introduced to ensure that all milk sold has a certain amount of vitamins A and D, since the child population is largely dependent on milk for these vitamins. Legislation of this type has already been introduced in Finland [241].

Fatty fish are a valuable and relatively cheap source of vitamin D. Of special value are fresh or tinned herring, bloaters, pilchards, kippers, sardines, salmon and eels. In 1989 the day's requirements of vitamin D could be bought as herring for three-halfpence, as tinned salmon for fourpence, and as eggs for tenpence [146]. Fish are also a good source of iodine for man, and of calcium and phosphorus [147] if their bones are eaten, as they may be in the case of sardines, sprats, and tinned herring and salmon. The bones of salmon have been found to have a high antirachitic value for animals [147]. It has been said that tinned salmon is the Londoner's sunlight. It is a pity the much cheaper herring does not hold this title, especially as the English Herring Fleet could land far more herring than are eaten at home [224]. In 1987 the average individual daily intake of vitamin D from herring, fresh or canned, was only 85 I.U. [225], though one fresh herring provides the day's needs of vitamin D.

Butcher's dripping bought in July in England is stated by Henry and others [168] to be as good a source of vitamin D as "summer butter," and, of course, it has a higher energy value than margarine.

Lindsay and Mottram [146] have explained how in the preparation of food additional vitamin D can be incorporated by the substitution of cod liver oil for olive oil or butter in such things as white sauce, mayonnaise, and batter when these are for fish dishes where the faint taste of the oil is masked by that of the fish.

Fortified Foods. Cowell [78] in 1925 first irradiated food as a means of increasing its vitamin D. In spite of the fact that this pioneer work was English, its commercial applications have been largely ignored in England while widely used in the United States.

Vitaminized margarine is practically the only food in England to which vitamin D is added; all margarine must contain, by law, 90 I.U. per ounce. The Ministry of Food itself refuses, in a personal letter, to explain whether by vitamin D is meant calciferol or the natural and probably more valuable vitamin D₃ (p. 655). In any case margarine can never be a true substitute for butter; the only important point is to prevent the public from believing it is. But since margarine instead of butter is an economic necessity in many families, high praise is due to the manufacturers whose own private work on vitaminization has enabled the Government to insist on it officially.

Milk is widely reinforced, especially in America. This can be done by (1) irradiating the cow, (2) irradiating the milk, (3) giving the cow vitamin D in her food, (4) adding vitamin D to the milk. By the first two methods vitamin D₂ is increased in the milk, as it is if fish liver oil is given in her diet, or is added later to the milk. But in both the latter cases fish oils may give a fishy taste to the milk. Vitamin D₂ is free from this objection and can be added directly to the milk or fed to the cow as irradiated yeast, giving "yeast milk." Irradiated milk generally contains about 185 I.U. per quart, while "yeast milk" and milk to which vitamin D is added generally contains 400 I.U. per quart. The great advantages of giving vitamin D in milk are discussed on p. 653.

Cheesc, or at least a substance which those with no palate could be deluded into thinking was cheese, can now be fortified with fish liver oils [242].

Concentrated Preparations of Natural and Artificial Vitamin D. There is complete disagreement about the relative values of calciferol, the artificially produced vitamin D₂, and vitamin D₃, the naturally occurring vitamin found in foods and fish liver oils. But it is significant that the controversy is entirely devoted to whether vitamin D₂ is as valuable as vitamin D₃: no proof is ever advanced that vitamin D₂ is more valuable than vitamin D₃. To our mind the evidence points to the superiority of the natural vitamin.

May and Wygant [19] in a study of four hundred and fifty-seven infants decided that while 200–500 I.U. of vitamin D given daily as cod liver oil were sufficient to prevent rickets, 400–800 I.U. of calciferol in oil were necessary to obtain the same effect. Hess and his collaborators [18, 71] from a study of sixty infants also give approximately the same figures. Jeans [18] concludes that "vitamin D of animal source appears to be more potent for the human being than the vitamin D of vegetable source (calciferol)." Brockman, Rietschel and others [20] state that vitamin D₂ is superior to calciferol when only one massive dose is given to protect children against rickets throughout the winter. Gerstenberger [186] comes to the same conclusion from experiments with monkeys. McBeath and Zucker [117] found the synthetic vitamin less valuable than the natural for increasing the immunity of children to dental decay (p. 689).

Morris and Stevenson [170], however, studying twelve children with active rickets came to the conclusion that vitamin D₂ and calciferol were of equal value when given in doses of 2,000 I.U. daily. Wilson [171] agrees with this from a study of thirty patients with osteomalacia or late rickets in India, who were given 10,500 or 21,000 I.U. weekly of either vitamin D₂ or calciferol. Himsworth and Maizels [172] also found no difference between the two vitamins when used in the treatment of a case of parathyroid tetany. Eliot and his collaborators [19], from their study of six hundred children in the United States, and Drake [19], in a prolonged study of over sixty Canadian children, agree that the antirachitic value of the two vitamins is the same.

Even if the balance of evidence, contrary to our own deductions from it, is held to prove that calciferol and vitamin D₂ are of equal value, there yet remain other objections to the use of calciferol. In animals calciferol

has been found more toxic than the natural vitamin (p. 647). Further, the greater antirachitic value of the natural vitamin for chickens increases over that of calciferol as the doses of the two are increased [69]. This suggests that for human beings the superiority of the natural vitamin might be enhanced at intakes above the minimum for preventing rickets. An analogy is found in the greater value of vitamin A than its provitamin (carotene) at levels of intake above the minimum for good growth.

Cod liver oil is the best concentrated source of vitamin D for supplementing the diet of both children and adults. Its advantages are: (1) It provides the natural vitamin D₃. (2) It provides vitamin A which, apart from its own great value, may enhance that of vitamin D



FIG. 162. Five weeks old chicks. The bird on the left received natural vitamin D₃ from cod liver oil, the bird in the centre received the same number of International Units of synthetic vitamin D₃ (calciferol), and the bird on the right received no vitamin D. The average weight and mortality of the ten birds in each group were: with the natural vitamin 399 grams and no deaths; with the synthetic vitamin 846 grams and five deaths; with no vitamin 259 grams and six deaths. (See also Fig. 161.)

[45, 188]. (3) It is nutritious. (4) A toxic dose of vitamin D cannot be given as ordinary cod liver oil owing to its bulk. This was emphasized by Thatcher [68] in his account of a child who died from overdosage of a concentrated oil. (5) The work of Jeans and Stearns [80] suggests that concentrations of vitamin D higher than those found in ordinary cod liver oil are not well absorbed. (6) Cod liver oil is of value apart from its vitamins, containing, for instance, traces of iodine, and a high proportion of unsaturated fatty acids [202] akin to those essential unsaturated fatty acids which sometimes are referred to as vitamin F (p. 785). (7) The cod liver oil requirements of England can be supplied by the English fishing fleet [224].

The traditional objection to cod liver oil is its taste. But poor children attending out-patient departments often ask for it, and one has the

impression that richer children largely dislike it because their mothers tell them it is unpleasant.

"Government cod liver oil *," which is issued free to pregnant women, infants and children, is a hotch-potch of vitamins D₂ and D₃ in arachis or pea-nut oil and fish liver oil; it contains roughly twice as much vitamin D as the minimum allowed in B.P. cod liver oil.

Concentrated fish liver oils can be used in those rare cases where ordinary cod liver oil cannot provide enough vitamin D, or where the taste is intolerable or the fat injurious as in coeliac disease.

Manufacturers of concentrated preparations of vitamin D should state on the bottle that there is a risk of overdosage if more is taken than is prescribed [223]. We have recently seen one patient who drank a preparation of calciferol in such quantities that he was showing the early signs of hypervitaminosis D (p. 696).

Effects of Cookery, Storage, Canning, Freezing and Drying on Vitamin D. Domestic cookery causes no loss of vitamin D, since it is not soluble in water nor easily destroyed by heat. Milk loses none by boiling or by pasteurization. Tinned and dried milks have the same vitamin D content as fresh milk, but the analyses of Bacharach and others [225] suggest that there is considerable loss when fish are canned or cured, since the average value of vitamin D in fifteen samples of fresh herring caught throughout the year was found to be 250 I.U. per ounce, and in five samples of canned herring only 50 I.U. per ounce. As, however, the fresh and tinned herring did not come from the same catch these differences may not be entirely due to the canning. Dried eggs lose a considerable amount of vitamins A and D when prepared by band drying but none by spray drying [243]. The value of butter is not impaired by its storage during transport over long distances, such as those between New Zealand and England [177].

AMOUNT OF VITAMIN D IN FOODS

This table has been chiefly compiled from the tables of Fixsen and Roscoe [154] and McCance and Widdowson [155].

The values for most of the fish, apart from salmon, lamprey and lampern, are only approximate, since research has been directed to the amount of vitamin D in the body oil of fish. To make this work of value in human diets, it has been assumed that the fat in the edible parts of the fish is body oil, and so since the amount of fat is known, the amount of vitamin D has been deduced.

Food.		International Units of vitamin D— probably D ₂ —in 100 gm. or roughly 3½ ounces.
<i>Butter.</i>		
Danish.	November–January	30–8
	February–April	8–14
	May–July	36–54
	September–October	44–15

* Cod Liver Oil Compound with added Vitamin D (Ministry of Food).

Food.	International Units of vitamin D— probably D ₂ —in 10 g. gm. or, roughly 3½ ounces.
Butter—continued.	
Dutch. General	21-48
English. November-March	10
April-June	15-40
July-August	55-97 (?)
September-October	40-15
New Zealand. General	30-57
Scotch. November-January	30-8
February-April	8-22
May-July	60-99
August-October	50-20
Dripping.	
English butcher's. July	33-44
Cacao Butter.	
	30,000 as D ₂
Cheese	
	Present. ? amount
Cream.	
English cow's	50
Eggs.	
Hen's. Yolks only. Summer	390
Winter	140
Whites only	0
Whole dried	220 (p. 657)
Duck's and other eggs	? as in hen's eggs
Fish.	
Cod	52
Eels	474
Herring. Canned, with Tomato	52-322
Canned, without Tomato	112-420
Fresh English, August	294-875
September	930-1,876
November	735
December	591-1,270
March	521
July	686
Kippers	590-744
Lampern	120
Lamprey. Sea	110-400
River	33
Mackerel	304-405
Oysters	5
Salmon. Canned. Chum and Chinook	200-800
Canned. Pink	600-700
Canned. Red	800
Sardine	1,800
Shrimp, flesh	147
Turbot	27

Food.	International Units of vitamin D— probably D ₂ —in 100 grm. or roughly 3½ ounces.
<i>Fish Liver Oils.</i>	
Cod	8,100–80,000
Cod-minimum allowed in English and American medicinal cod liver oil	8,500
Cod Liver Oil Compound with added vitamin D (Ministry of Food)	20,000 as D ₂ and D ₃ in Fish and Arachis Oils.
Halibut	20,000–400,000
Tunny (various kinds)	1,600,000–25,000,000
<i>Fungi.</i>	
Edible	83–125 as D ₂
Mushrooms. Grown in dark	21 as D ₂
Grown in light	63 as D ₂
<i>Green Vegetables.</i>	0
<i>Liver.</i>	
English. Calf	0
American. Calf	10
American. Lamb	20
American. Ox	40–50
American. Pig	40–50
<i>Margarine.</i>	
Vitaminized	210. Kind of vitamin uncertain (p. 654)
Unvitaminized. Vegetable	0
<i>Milk.*</i>	
Human. Town. Summer	6.2
Human. Town. Winter	5.9
Human. Maximum possible (p. 660)	10
Cow's. Summer	2.4–3.8
Winter	0.3–1.7
Irradiated milk	Generally standardized at 12.
Yeast milk	Generally standardized at 85 as D ₂ .
Fortified milk	Generally standardized at 85 as D ₂ or D ₃ .
<i>Vegetable Oils.</i>	
Arachis or Pea-Nut	0
Olive	0

HUMAN REQUIREMENTS OF VITAMIN D

The dietetic requirements for vitamin D given in the following pages are for the inhabitants of temperate climates who are receiving little irradiation from the sun. During the summer, especially in the country, less vitamin D need be taken in the food. It is assumed that the diet is not

* In 100 c.c. or roughly 3½ fluid ounces.

grossly abnormal, especially for calcium and phosphorus, and that no liquid paraffin, or laxatives containing liquid paraffin or mineral oils, are being given since these decrease the absorption of vitamins A, D, K, and probably E (p. 718).. Such laxatives should never be given, especially to infants.

The British Pædiatric Association in 1942 stated that infants, children and mothers require the following amounts of vitamin D daily, expressed in international units, though these amounts, in the light of the work discussed below and of the recommendations of the U.S.A. National Research Council, are probably excessive, especially for premature infants.

Premature infants (under 5½ lb. at birth) 1,400
Full term infants 700
Children, 5-14 years 500
Puberty and adolescence 300-600
Adults, male and female 300-600 ?
Pregnancy and lactation 700
Old age 300 ?

Breast-fed full term babies often develop rickets. Premature babies and those which grow very rapidly generally do so unless they are given extra vitamin D. If surprise is felt that breast milk should ever be inadequate, it must be remembered—quite apart from the mother's diet being often deficient in the vitamin—that children should gain vitamin D from the sunlight on their bodies; their milk probably only supplements their sunlight rather than the other way round. Cow's milk or other artificial foods always require additional vitamin D. The problem is to decide how much vitamin D infants require, not only to avoid rickets, but also to give the best rate of growth (p. 645).

Human milk from women in towns was found by Drummond [79] to contain on an average about 68 I.U. in a quart, though some milk had little more than 40 I.U. Values could be increased to 114 I.U. if the mother supplemented her own diet with 200 I.U. of vitamin D. Any further increase in the maternal intake did not further raise the vitamin in the milk, which suggests 114 I.U. is its best content. This gives the infant in the first few months of life less than 100 I.U. a day, but its value of course is enhanced by being given in milk (p. 653) and by acting with the ideal calcium-phosphorus ratio for human infants. Polskin and others [244] report that when women take about 40,000 I.U. daily their colostrum contains 104-247 I.U. per quart and their milk, during the first nine days of lactation, 142-500 I.U. per quart. Of course such a high intake of vitamin D is entirely abnormal and the amount in the milk may be rather an overflow than a physiological secretion.

Drummond's figures agree closely with those of Jeans and Stearns [80], who found that for three months old infants 70 to 100 I.U. given in cow's milk protected them against rickets.

Growth, however, is not stimulated to its maximum by doses which just prevent rickets [78, 81]. Whether the greatest possible growth is to be desired has been discussed on p. 645. But whatever the answer it seems that it is wise to give more vitamin D than will just be protective,

partly because if the infant suddenly grows rapidly it will no longer be protected, and partly because the figures given above appear to be those which an infant naturally receives only from its milk, unsupplemented by the considerable amount which should be provided by the action of the sun on the skin. The latter under modern conditions is so small that it must be compensated for by an increase in the vitamin D in the diet. The maximum amount of growth is given by 300 to 600 I.U. a day [78, 81], while doses of 1,800 I.U. definitely reduce the rate of growth [59] and delay dentition [168]. Barnes [209] also considers that 600 I.U., provided by fish liver oils from whatever fish, is ample, since forty-eight infants with rickets were cured as rapidly with 600 as with 900 or 2,400 I.U., cure being assessed by blood phosphatase estimations. Corner [204], in her very careful investigations on town children living at home, found that a supplement of 400 I.U. daily had no effect on the incidence of rickets, which she considered meant either that this amount is inadequate or that it cannot overcome the other contributory causes of rickets among poor town children; this is largely confirmed by Krestin [207], who reports that the free distribution of "Government" cod liver oil,* containing 400 I.U. per daily dose, to pregnant and nursing mothers and to infants had no beneficial effect. But it must be remembered that both these observers were dealing with the poor and so could not be certain the oil was being taken regularly. This is a strong argument in favour of providing free cod liver oil with a higher content of vitamin D than would in theory be necessary in order that when a dose is forgotten it is compensated for by the next dose.

From the clinical point of view, therefore, breast-fed infants should be given two small teaspoonfuls of an ordinary cod liver oil a day; this will contain about 500 I.U. For artificially fed infants another teaspoonful should be given. The oil is started about a month after birth in 5 drop doses thrice daily, dropped on the tongue, and increased slowly to the full quantity in about three months.

Premature infants suffer from two drawbacks—they grow very fast and they have not stored the bone minerals which are acquired in large quantities in the last weeks of foetal life. Faced with a hesitant digestion, enough minerals can seldom be absorbed for some months, so that slight osteoporosis is inevitable, but this can be improved and rickets avoided if relatively large doses of vitamin D are given. After the first month cod liver oil should be taken in 5 drop doses thrice daily and rapidly increased if it does not cause indigestion. The oil can be dropped on the tongue. More concentrated preparations can be tried, but they are probably less well absorbed (p. 656). Not less than 600 I.U. appears to be the dose which should be aimed at as soon as possible [82, 88, 192, 209]; the 1,400 I.U. advised by the British Pædiatric Association would appear to be large enough to check growth and dentition [59, 168]. Mixing the oil with the milk in the feeding bottle is unsatisfactory, since it tends to float to the top and stick to the sides of the bottle.

Massive single doses of vitamin D are sometimes given to infants to confer prolonged protection against rickets both in Germany and in

* Cod Liver Oil Compound with added Vitamin D (Ministry of Food).

America. It is impossible not to hesitate in suggesting that this form of treatment could be wisely introduced into English medicine. The advocates of the single massive doses claim that it is safe, that the time of the mother is not wasted by frequent visits to baby clinics to obtain cod liver oil she cannot afford to buy, and that the infant of the most feckless mother can be protected for several months against rickets, though he is only brought to a clinic once. Such arguments, granting the dubious assumption discussed below that one huge dose of vitamin D is not toxic, are only valid in backward countries in which, unlike England, there are few doctors and the poor are not educated to bring their infants regularly to clinics. In England every effort should be made not to curtail the number of visits to infant welfare clinics, but to increase them. Rickets is now one of the least of the ills against which the slum child needs protection: the value of a clinic lies not in doling out free cod liver oil, but in guarding the health of the healthy infant by advice and those regular and frequent examinations which alone can forestall illness. So in England one massive dose of vitamin D will not excuse regular attendance at a clinic nor even save the trouble of giving the infant cod liver oil since this is still needed for its vitamin A and its very valuable fat.

The safety and also the value of single massive doses of vitamin D has not yet been properly investigated, with adequate controls, to show whether or no there is any impairment, at least for a short time after taking the vitamin, of growth, of calcification, of dental development and of renal function. Jeans and Stearns [59] and Speidel and Stearns [168] have shown that both growth and dentition are delayed by 1,800 I.U. of vitamin D daily and Türk's observations [20] suggest that the unsophisticated kidney of the infant is more liable to be damaged by infections occurring just after a large dose of the vitamin. There is general agreement that impaired renal function is a reason for not giving large doses of vitamin D, but to diagnose this in an out-patient clinic is difficult. It is also hard to believe that the infant has any adequate mechanism for dealing with amounts of vitamin D far larger than any it could obtain from its food or by the action of sunlight. However, it must be admitted that none of the several hundred German and American infants who have been given a single dose of 600,000 I.U. have shown any obvious toxic symptoms, though these are common when *daily* doses of 200,000 I.U. or less are given to adults.

Germany was the first country to use single massive doses of vitamin D both for the prevention and the cure of rickets, Harnapp [84] coining the phrase "Vitamin D-Stoss" therapy for this. From a study of one hundred infants he decided that protection against rickets for at least four months was given by 300,000 to 600,000 I.U., though thirteen of sixty infants given 600,000 I.U. by Brockman [20] developed craniotabes within four months. Türk [20] prevented rickets in thirty premature infants or six months by oral or intramuscular doses of 200,000 to 400,000 I.U., while most of his untreated controls did develop rickets. In America Rambar and others [226] successfully treated twelve infants with a single oral dose of 600,000 I.U. or a monthly dose of 100,000 I.U. for seven months; no controls were studied and their results were only confirmed by X-rays.

Wolf's first observations [227] on the effect of large oral doses, were partly controlled, since of his sixty-two infants, eighteen had rickets at the beginning of treatment and none had rickets nine months later, after having had two doses of 600,000 I.U., with a three to six months' interval between. In a second paper, Wolf [227] advises 50,000 I.U. at one and two months of age and a third dose of 600,000 I.U. at three months of age. Of twenty-one infants given these doses, two developed rickets, radiologically confirmed, just before the third dose—and this at an age when X-rays are more prone to miss than to disclose rickets. As Wolf ignores craniotabes and mild beading of the ribs, without positive X-rays of the wrists, the incidence of rickets in his cases may be considerably higher than he states. He also includes breast-fed babies in his series "because it has been shown that vitamin D is not secreted by the breast."

Krestin [257] is the only English worker who has used single massive doses. He gave 800,000 I.U. by mouth to forty-three infants under one year of age. Of these three developed rickets within six months. In a comparable group of fifty-six infants, whose mothers were supposed to be giving them 1,500 I.U. daily, five developed rickets. The single massive dose had no adverse effect on growth, gain in weight or liability to infection.

"Activated" ergosterol has been used by American workers when giving single massive doses because of the erroneous belief that when ergosterol is vaporized and then "activated" by an electric discharge—that is, when vitamin D is made by the Whittier process—it is non-toxic, due to the absence of those substances which are formed when ergosterol is irradiated. But "activated" ergosterol is virtually as toxic as the irradiated, causing all the usual symptoms of toxicity—even in one adult in doses as low as 25,000 I.U. daily [228].

Children between the ages of two and twelve should have two teaspoonfuls of cod liver oil in the winter when sunlight is scarce. If the parents have already warned the children it is unpleasant or if the children genuinely dislike the taste, the classical method of floating it on peppermint is excellent, or of taking a pinch of salt on the tongue before the oil. Concentrated fish liver oil capsules may be taken as a last and expensive resort. Of course ultra-violet irradiation can be given instead of cod liver oil, though with more trouble and cost (p. 652).

Boarding schools—both boys' and girls'—often give very bad diets, especially from the point of view of the costly vitamin D at ages when it is most needed. In examining children who live at a boarding school for two-thirds of the year, the probability of cod liver oil being needed must be remembered (p. 658).

Puberty and adolescence, with its sudden growth impulse, especially needs cod liver oil, since mild rickets or osteoporosis (Fig. 168) may occur at this period.

During pregnancy and lactation vitamin D is required both for the mother and for the child. The results of a deficiency are well seen in osteomalacia. McLennan [245] suggests that mild osteomalacia may explain why some women who have had no difficulty in earlier labours may appear in later ones to have a slight contraction of their pelvis.

Children of mothers deficient in vitamin D have been reported to have unduly soft skulls, poorly calcified bones [86] and to develop rickets, or even to be born with rickets [101, 102]. While Drummond [79] found that 200 I.U. daily in the maternal diet gave the maximum possible amount of vitamin D in the milk, other studies have shown that the mother requires larger amounts if she is to preserve her own mineral metabolism [87, 88,

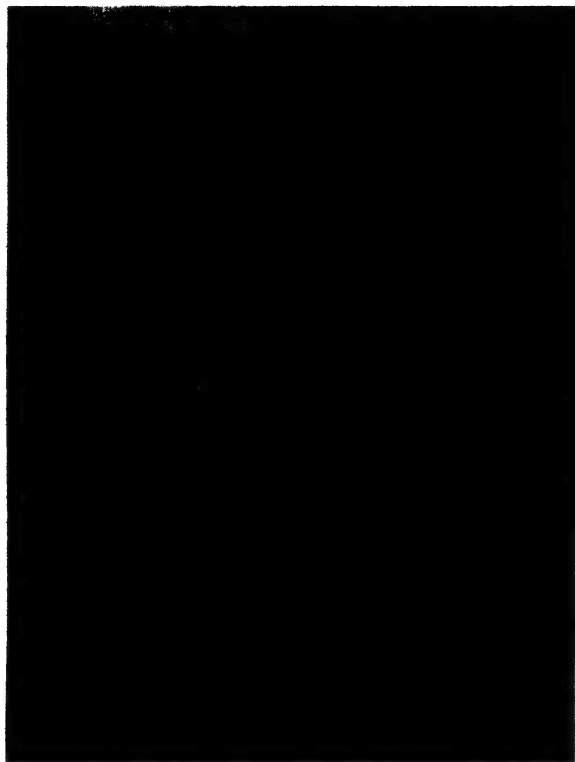


FIG. 168. A severe case of adolescent rickets in England.

89]. During pregnancy and lactation about 600–800 I.U. daily appear to be ample.

The nausea of pregnancy may force the use of concentrated fish liver oil capsules. Concentrated preparations of the other vitamins will also be required, since pregnancy increases their requirements, while nausea decreases their intake in food. Theobald [148] investigating the toxæmia of pregnancy gave to fifty women calcium and vitamins A and D, the latter in doses of 450 units daily. Compared to a control group of fifty women the incidence of toxæmia was more than halved. Finola [149] and others give the warning that too much vitamin D may cause excessive calcification of the foetal bones of the head making birth difficult. It is also interesting to note that claims are made for the use of vitamins B₁ and E in the treatment of pregnancy toxæmias (pp. 266, 745).

The adult needs are uncertain—most adults apparently get enough from their food and the sun to prevent obvious deficiencies. But it seems probable that most diets lack some vitamin D: breast milk only reaches its maximum vitamin content if the mother takes about an extra 200 I.U. daily [79]; while senile osteoporosis is fairly common and appears to be due to a mild vitamin D deficiency [85]. Reed and his collaborators [45] have reported that huge doses—150,000 to 200,000 I.U. daily—given to men doing sedentary work increased their general well being, weight,



FIG. 164. Fetal rickets in a Chinese baby photographed on the day of birth. Note the rickety rosary and vertical grooves. (See also Figs. 159 and 166.)

and muscular power. The men previously had been taking only about 100 I.U. daily, and most were underweight before the investigation started. Of course 200,000 I.U. a day is a fantastic amount if it is taken in the food, and it seems unlikely that "in a state of nature" irradiation would enable the body to form so much. The doses employed were, in fact, unphysiological, but this does not detract from the implication that more vitamin D is desirable in the adult's diet. Probably 800 to 600 I.U. a day should be taken.

In old age the value of sunlight is often lost, since the old keep indoors,

and the appetite may be small. According to Mèulengracht [85] supplementing the diet of the elderly with cod-liver oil should prevent osteoporosis and the brittleness of the bones which so frequently leads to serious fractures, which, in the femur, for instance, may end fatally from pneumonic complications.

DISEASES DUE TO DEFICIENCY OF VITAMIN D

RICKETS

Lack of vitamin D is the cause of rickets (see Frontispiece).

The grotesque deformities of the rachitic dwarfs of a century ago are now so rare that one is apt to forget that mild rickets is still one of the commonest deficiency diseases. Probably between one-third and one-half of all the infants and children in England have or have had rickets. Reports on the incidence of rickets give such different figures because the different authors have based their diagnosis on different criteria. Rickets is now so mild a disease that its clinical recognition is often difficult, especially after infancy. This has lead not only to an unjustified belief that clinical reports on the frequency of rickets are quite valueless, but also to undue reliance being placed on radiological reports. Actually the only completely reliable reports are those based on the level of the serum phosphatase and confirmed by clinical or radiological or post-mortem examinations. Such confusion, however, now exists about the incidence of rickets that some recent reports need to be discussed in full.

X-rays are not so valuable as is generally believed. They were the basis of the widely quoted survey on rickets, published in 1944, which was carried out in Great Britain during the first two months of 1943 on 4,818 urban and rural infants between the ages of three and eighteen months by the British Pædiatric Association for the Ministry of Health [208]. Each infant was examined clinically and also had one wrist X-rayed. Clinically thirty-three and one-third per cent. of all the infants had active or healed rickets, while in some towns the figure was above sixty per cent. These figures were, however, completely ignored when assessing the results of this investigation on the grounds that they were quite unreliable both because only five per cent. were confirmed by X-rays and because half of the 1.7 per cent. of infants who had positive X-rays were not diagnosed clinically as having rickets. But mild rickets in infants shows itself only in the skull and the costochondral junctions, the wrists not beginning to be affected until toward the end of the first year. Since only the wrists were X-rayed most of the younger infants and many of the older inevitably appeared to be radiologically normal, however clinically definite was their craniotabes or the beading of their ribs. In the final summary no attention was drawn to such considerations nor to the uselessness of the figures which were given. "The (calculated) rate of incidence of rickets diagnosed radiologically for children between three and eighteen months of age was two and a half per cent. before six months of age, four per cent. during the first year of life, and negligible over this age." The clinical finding that thirty-three and one-third per cent. of infants had active or healed

rickets is, on the other hand, probably correct, agreeing with the other reports discussed below and most of the investigations made during the last ten years; these are summarized in the report which has just been discussed.

An analogous investigation to the above was carried out by Krestin [207], who during the autumn, winter and spring months of 1942 and 1948 examined clinically and radiologically one thousand healthy children between the ages of two months and five years who were in nursery schools in Preston. Of five hundred and eighteen infants below the age of two years 11.0 per cent. were diagnosed as having active rickets by X-rays and 86.5 per cent. by clinical examination. After the age of eighteen months rickets steadily declined till between the ages of four and five years it was clinically present in only 16.4 per cent. of seventy-seven children. The post-mortem examinations discussed below suggests a higher incidence in this last age group and also confirm the uselessness of X-rays for the diagnosis of mild rickets.

Estimations of plasma phosphatase [151, 204, 205, 208, 209] probably give the most reliable figures for the frequency of rickets. The outstanding work on this subject, and also on many aspects of the diagnosis and contributory causes of infantile rickets, is that of Corner [204] who in 1944 published the results of her investigations on infants in Bristol which lasted from September 1939 to May 1941. Eight hundred and twenty infants between the ages of two months and two years, being a representative sample of the healthy and sick population, were examined clinically and the plasma phosphatase was estimated in seven hundred and ninety-seven. X-rays and post-mortem examinations were also carried out in a few cases. Rickets was not diagnosed unless the clinical findings were confirmed by an increase in the plasma phosphatase or by X-rays or by autopsy. Definite rickets was present in 31.4 per cent. of all the infants and a further 12.2 per cent. showed doubtful or very mild evidence of rickets. At six to nine months of age 52.0 per cent. of infants had rickets and at twelve to eighteen months 48.0 per cent. Below six months the diagnosis was definite in only 26.2 per cent., but in another 17.8 per cent. the serum phosphatase was either definitely raised or slightly raised and accompanied by doubtful clinical signs.

Clinical diagnosis used alone for assessing the frequency of rickets in infants under two years of age has already been discussed, while reviewing surveys based on other forms of diagnosis. The British Pædiatric Association Report [208] gives the percentage of infants affected as 33.3, Krestin [207] as 33.6 and Corner [204] as 45.9. The close agreement of these figures and, especially, the agreement of Corner's clinical finding of 45.9 per cent. with her confirmed finding of 31.4 to 48.6 per cent., does most strongly support the validity of investigations on infants based solely on clinical observations.

Post-mortem examinations for the estimation of the frequency of rickets have only, in recent years, been used by Follis, Jackson, Eliot and Park [206] who in 1948 published the results of consecutive post-mortem examinations on two hundred and sixty children between the ages of two and fourteen years who died from whatever cause in the Harriet Lane

Home of the Johns Hopkins Hospital in America. Histological studies, the validity of the technique being carefully confirmed, were made of the shafts and costochondral junctions of the middle ribs. In these two hundred and sixty children active rickets was present in roughly forty per cent. at all ages between four and fourteen years, and in sixty per cent. during the third year of life. Half the children of this whole series who died from an acute illness, lasting at most two weeks, had rachitic changes which were too extensive to have developed during their illness. So it seems probable that rickets is as common in healthy as in the sick children.

It is of considerable interest that X-rays were taken of all the ribs which were removed and only five were positive though one hundred and seven showed, histologically, active rachitic changes: the five positive X-rays were all of cases below five years of age.

The importance of very mild rickets, such as that dealt with in the above surveys, may be thought to be slight since nearly half the healthy adult population of England must have had rickets and yet do not appear to have permanently suffered. But mild rickets is of great importance. Firstly it is a sign that our infant and child population is still badly nourished. Secondly the rachitic infant is one who is apt to develop tardily, is prone to infection, is not going to grow to his full stature, and in adult life will join that mass of patients whose chests and legs are mildly deformed, and whose pelvises are sufficiently contracted to make child-birth difficult. In fact, if the importance of the diagnosis and treatment of very mild rickets is not immediately obvious, yet taking the long view it is of the greatest value.

Contributory Causes of Rickets. In temperate climates the lack of sun and the expense of food rich in vitamin D often cause the milk of nursing mothers and the diet of children to be deficient in vitamin D (p. 652). It is not, therefore, surprising that the time of year at which rickets is most common depends entirely on the amount of sunlight. Thus rickets shows a sharp increase in late winter and early spring when the reserves of vitamin D are low both in nursing mothers and in food, and have not yet been replenished. Rickets, however, may occur with ample sunlight especially in dark-skinned people suffering from severe malnutrition [114, 205].

An inherited disposition to rickets is generally accepted, though, of course, accurately to disentangle all the factors which ameliorate or intensify the disease in man is impossible [101]. Still [56] points out that sometimes the osseous, sometimes the nervous or respiratory systems are most affected in early rickets, this predilection of the disease for a special system often being found in all the children of the same family. It seems probable that the negro requires more vitamin D than natives of northern countries [90, 206].

Turning to the individual infant, by far the most important personal factor in the causation of rickets is the rate of growth. It is so important that the position has been summed up as "no growth, no rickets."

Breast feeding during the first six months of life reduces the incidence of rickets but increases it after this age compared to infants weaned on to a good diet. A poor antenatal diet has an adverse effect which lasts at

least until the end of the second year, this prolonged effect being probably due to the antenatal diet reflecting the diet which is given to the weaned infant. It makes little difference whether artificially fed babies are given milk, or dried milk or sweetened condensed milk [204].

Premature infants and twins are very prone to develop rickets, partly because they grow fast, partly because they start life deficient in bone salts and vitamin D, with a digestion which cannot easily make up the deficiency.

Infections, chronic and acute, are generally believed to make rickets worse. However it is an old observation that children with tuberculous glands seldom develop rickets [101] and in Corner's study [204] of eight



FIG. 165. Five French children in one family with rickets, aged $6\frac{1}{2}$, $11\frac{1}{2}$, $18\frac{1}{2}$, $14\frac{1}{2}$ and 17. Note bossing of the forehead, bent tibiae, curvature of the spine, and protuberant abdomen. (See also Fig. 156.)

hundred and twenty infants rickets, which was present in 81.4 per cent. of these, was commonest in the healthy and lowest in theseverely ill. In post-mortem studies on children, between two and fourteen years of age, rickets was not found associated with any particular disease apart from lead poisoning [206]. Presumably the healthy infant is more prone to rickets than the sick because he grows faster.

Congenital syphilis is frequently associated with rickets, or, rather, causes or exaggerates the deficiency. Craniotabes, which for so long has been regarded as either rachitic or syphilitic in origin—if not due to osteogenesis imperfecta or hydrocephalus—is discussed on p. 672. There it will be seen that probably it is purely a rachitic condition, though it may be an example of the predilection of an infection for certain tissues

being determined by the especial susceptibility of such tissues to a deficiency of a particular vitamin, such as is seen in the occurrence of bronchitis in infants suffering from lack of vitamin A.

The age of onset of rickets varies slightly in different countries, due most probably to the maternal diet, which starts the infant off either with an adequate or poor supply of vitamin D and bone salts. In the post-war famine in Vienna where the mothers themselves were poorly nourished, rickets in infants only three months old was a common finding, while in



FIG. 166. Fœtal rickets in a Chinese baby, X-rayed on the day of birth. Note the uneven frayed diaphyseal ends of the radius and ulna, and the cupping of the latter. (See also Figs. 159 and 164.)

England rickets before the age of six months is generally thought to be fairly rare, though this is not confirmed by Corner's figures which have been given in the previous discussion on the incidence of rickets (p. 666). After six months rickets increases until the middle of the second year and then declines as the first wave of the growth impulse is spent and the child tends to eat a wider diet and to spend more time out-doors.

Rickets, however, can occur at any age. Infants (Figs. 164 and 166) have been born with rickets [101, 102], and "late rickets" or "juvenile osteomalacia" may occasionally be found in older children (Fig. 163),

especially during periods of rapid growth, such as at the second dentition and puberty; a wartime example in England is described on p. 658. At this age the picture hovers between rickets, osteoporosis and osteomalacia, depending on how much active growth is still going on, the minerals in the diet, and the severity of the deficiency (Fig. 168). After growth has finished a deficiency of vitamin D causes osteomalacia, which is only adult rickets, modified by the absence of growth (p. 688). Girls are slightly more susceptible to rickets than boys, and dark children more than blond [101].

The General Symptoms of Rickets. The clinical picture of rickets is too often painted as if the bone changes alone were important; but rickets is a disease which affects the whole body so that even if the osseous system were ignored a very definite disease picture would still remain.

The rachitic infant is a tired, restless, unhappy creature; by day he is fretful, by night he sleeps badly, throwing off his bedclothes as he twists and turns. He impatiently rolls his sweating head on his pillow, even to the extent of wearing away his hair. His bowels are often constipated with sudden attacks of diarrhoea. His stomach is distended, partly because the intestines lack tone and are blown up by carbohydrate fermentation from his faulty diet, partly because the liver and spleen are forced down by the deformed chest, and partly because the abdominal muscles are flabby. This flabbiness affects all the other muscles of the body while at the same time the ligaments of the joints are lax, so that the limbs may be twisted into bizarre positions. The Buddha-like position commonly seen in statues of the holy men of the East, with the soles of the feet lying upwards, is probably the result of rickets and osteomalacia. A very mild tenderness of the bones is common, causing the child to cry if roughly handled. But any marked tenderness is more suggestive of scurvy.

Catarrhal infections are common; they are not due to the rickets, but rather to the unhealthy life and poor diet of these children, a diet which, among other things, is probably grossly deficient in vitamin A.

Spasmus nutans is a rare complication: when it occurs it is generally with mild rather than severe rickets. The child slowly nods or shakes his head when it is unsupported, and at the same time has a rapid, very slight or most obvious nystagmus, which is almost unique in often being unilateral. When the two eyes are affected their movements may bear no relation to each other and move in any direction and manner. Hippus—rhythmic contractions of the pupil—is also sometimes seen. Spasmus nutans is generally found in the first year of life, starting in the winter, and disappearing in a few months. Its cause is obscure. It is not due to the infant living in the twilight of a cellar or dark room, such as would cause miner's nystagmus, because it is found in infants who live in well-lit rooms.

Anæmia is common, being generally of the normocytic type; it seems to be rather the result of general ill health or bad food than an essential part of rickets, though in animals vitamin D, especially from fish liver oils, slightly increases the absorption of iron and the formation of hæmoglobin [251]. The liver and spleen may be just palpable. This has no significance, being due to the deformed thorax pushing down the viscera. A really enlarged spleen suggests syphilis. Von Jaksch's anæmia, or

pseudo-leukæmia infantum, is often associated with rickets for some still obscure reason.

The usual infantile milestones are passed late. The teeth are often not cut until after the first year (p. 689), crawling and walking are delayed, or if walking has started the onset of rickets "takes the child off his legs."

Mental development is unaltered, though severe cases may show the precocity which is common in invalid children who of necessity are too much in the company of adults.

The Bony Changes of Rickets. The bony changes which may occur in rickets are craniotabes, a large square-shaped head, late closure of the fontanelle, beading of the ribs and deformity of the chest, bending of the long bones, enlargements at the epiphyses, and fractures.

To understand these changes it must be remembered that :—

(1) Rachitic changes are greatest where growth is most rapid.

(2) Deformities are the result of the combined action, on the bones, of gravity and the pull of muscles.

Craniotabes is the earliest finding in rickets and is diagnostic of the disease when associated with beading of the ribs [204]. While it is very common in rachitic infants under nine months, it is often completely absent in severe cases in older children. This is partly because the growth of the skull is extremely rapid in the first months of life, and partly because when children start to sit up there is no longer continuous pressure from the pillows on the skull.

In typical craniotabes round or oval unossified areas are found in the skull which yield like parchment under the pressure of one's fingers, giving a crackling feeling. These areas vary in size from those which can be just felt with the tip of a finger to those which appear to fit the entire ball of the thumb. They occur in the bones on which the weight of the head rests as the infant lies in his cot; the upper occipital, the posterior parietal, and sometimes in one upper temporal bone if the infant lies much on one side. They do not join with the sutures, but often lie close to them.

Confusion may arise either from the soft feeling of the skulls of some infants in whom calcification is delayed, or because craniotabes is generally stated to be a finding common not only in rickets, but also in congenital syphilis. Probably craniotabes is always rachitic in origin, so that when it occurs in syphilitic infants they are suffering from two diseases. Still [56] points out that the condition in syphilitics will clear up in seven or ten days with vitamin D and no anti-syphilitic treatment, and also that craniotabes is frequently associated with laryngismus stridulus, which is a rachitic and not a syphilitic condition. Also syphilis tends to make the bones harder, not softer. In osteogenesis imperfecta the whole of the vault of the skull, and not only small areas in its posterior half, is largely membranous, while in hydrocephalus the size of the head prevents confusion.

The persistence of the anterior fontanelle is common and should suggest rickets if it still measures an inch each way at the end of the first year, or persists after the age of two.

The enlargement of the skull is difficult to explain. The four crosses

over the frontal and parietal eminences which give the square hot-cross-bun effect are due to the heaping up of osteoid tissue in these regions.

The chest also suffers in early infancy when the ribs are growing rapidly and are very soft. The pull of inspiration drags in the sides of the chest, so that the sternum is left sticking forward like the keel of a boat. At the same time the lower ribs flare out, being supported from collapse by the liver and heart, leaving "Harrison's sulcus" above them. These deformities of the chest are serious. Full expansion of the lungs is impossible, so that even mild catarrhal infections are liable to end fatally



FIG. 167. Rickets in an English infant. Note the deformity and enlargement of the epiphyses at the wrist. (See also Fig. 168.)

because the flaccid cage of the ribs collapses more and more with any obstruction to inspiration.

Beading of the ribs, the "rickety rosary," is a nearly constant finding after the age of six months or even earlier, but not one on which much reliance as a diagnostic sign can be placed in mild cases. Corner [204] in her extensive study of eight hundred and twenty infants found that one-third of all infants with mild beading and one-eighth with definite beading had not got rickets, judged by the level of the plasma phosphatase; beading associated with craniotabes was diagnostic. The "beading" is the expanding osteoid tissue at the junction of the rib and its cartilage, being most obvious over the fifth, sixth and seventh ribs. Some slight

swelling at the costo-chondral junctions in young infants is normal, and has no rachitic origin. The ribs in severe cases may be displaced backwards, so that no beading is felt. "Posterior beading" is caused by greenstick fractures at the angles of the ribs.

The arms become deformed when the child starts to crawl or sit upright. In the latter position he appears to fall into himself, and as the weak muscles of his back cannot hold his back straight, he curls forward supporting himself on his arms. This causes them to bow under the

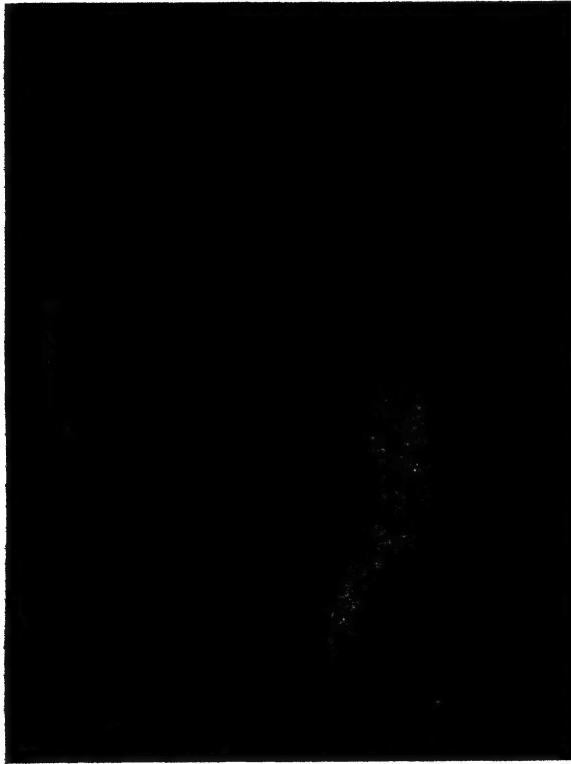


FIG. 168. Rickets in an English infant. Note the bowing of the tibiae, and the enlargement and deformity of the epiphyses at the ankles. (See also Fig. 167.)

strain, while the already enlarged radial and ulna epiphyses broaden still further. The clavicle is bent upward and forward at its inner third by the pull of the neck muscles at one end and the drag of the arms at the other. The fingers sometimes show sausage-like enlargements due to the shafts of the phalanges swelling from osteoid tissue, while the joints remain a normal size.

The femur develops a forward curve from the weight of the legs when the child is sitting in his mother's lap, while later on, if he manages to walk, the lower third of the tibia tends to bend outward and forward

and the femur to bend outward. But at this stage any deformity of the legs may occur, increased by the lax ligaments of all the joints.

Pelvic distortion, is, however, the most serious result of rickets. It may make child-bearing in later life impossible except by Cæsarean section, so that in girls every effort must be made to insist on thorough treatment, and above all complete rest from walking until the pelvis is firm enough not to bend under the weight of the body.

Greenstick fractures are common and may be overlooked, being often painless and giving no more deformity than may have already arisen from the twisted bones.

Dental caries is not caused by rickets (p. 639).

Atrophic rickets is the name given to rickets where osteoporosis is profound, due to the diet being grossly deficient in calcium as well as vitamin D. It is commonest in the first year of life, and in premature babies.

Hypertrophic rickets occurs in older children, especially when the rickets is mild and chronic. It causes great thickening of the bones due to masses of osteoid tissue in which only some slight calcification can be seen.

Spasmophilia. The nervous symptoms of rickets are so important that they are often discussed as if they were a different condition, and it is true that some of the worst cases, those with generalized convulsions or prolonged attacks of tetany, show only very slight signs of the underlying rachitic condition. Indeed, it may be the convulsions which first cause a suspicion that rickets is present.

The proportion of rachitic children who suffer from spasmophilia varies greatly in different places. The underlying mechanism is a low blood calcium; where the calcium of the diet is high the tendency to spasmophilia should therefore be slight, but as yet this has not been investigated. Boys are affected more often than girls and the condition may occur at any age, even in infants a few weeks old.

The irritability of the nervous system may show itself in many ways.

The most trivial manifestations are sudden starts with a cry as if the child were suddenly pricked; or for a moment he turns his eyes up; or he cries out in his sleep, rolls over and is quiet again. Facial stiffness is also quite common. The muscles of the child's face are in spasm, so that when he cries the face gives an impression of being stiff. Change of expression is limited, lacking the finer shades.

Or, again, true tetany may occur, the hands and feet being drawn into the typical position, with the thumbs pressed against the fingers, which are held rigidly straight though flexed at the carpo-metacarpal joints with the wrist slightly bent. The feet are extended while the toes are bent down and bunched together. These spasms may last for seconds or hours. They appear to be only painful when they start, so that children will often go on clumsily playing with their toys during an attack. If the spasm lasts for some hours both the hands and feet may swell from static congestion.

Laryngismus stridulus is, however, a more frightening symptom. The child on waking, or when he starts to cry, or indeed for no very

obvious cause suddenly gets a spasm of his glottis. He holds his breath, appears terrified, and goes blue in the face. Then suddenly the spasm passes and he draws in his breath with a long whoop. Sometimes the child does not recover so simply but goes into general convulsions.

General convulsions may occur for no reason, but usually the child has had an attack of diarrhoea, or a mild feverish upset. The attack starts with the child going into tonic stiffness which passes into clonic convulsions. After a minute or two the attack passes, the child being again normal but exhausted. Generally these attacks occur at long intervals, but they may follow each other so rapidly that the child does not regain consciousness between each one.

There is a definite danger to life in infantile convulsions so that it is important to recognize and treat the early mild symptoms of spasmophilia



FIG. 169. Tetany in an English infant with rickets. Note the position of the hands, and their swelling from static congestion.

before generalized convulsions occur. The treatment is discussed on p. 681.

Examination of the child may disclose any of the classical signs of latent tetany due to an unusual excitability of the motor nerves—Chvostek's sign, or twitching of the muscles of the face when the facial nerve in front of the ear is lightly percussed; Trousseau's sign, or compressing the arm or leg by encircling it with the hand and squeezing, when the hand or foot goes into the position assumed in attacks of tetany; Erb's sign, or undue excitability of the motor nerves to electrical stimulation—but the necessity for electrical apparatus and experience in its use vitiates the value of this test.

Other causes besides rickets may cause infantile convulsions. Acute fevers and infections of the brain and spinal cord often start with convulsions. In these conditions the child between its convulsions is still ill while in spasmophilia it is well. Trivial causes like constipation or indigestion may cause doubt for a moment, and middle ear disease must

be remembered. Any infection may flare up a latent tendency to true spasmophilia.

Idiopathic epilepsy is rare at this age, and will not yield to the treatment for spasmophilia.

Lead encephalopathy may closely simulate spasmophilia, the earlier symptoms being vomiting and convulsions. Mild cases often occur in the spring when the increased supply of vitamin D from sunlight enables children to absorb more lead from the paint of their toys, etc., since lead absorption is increased by vitamin D [158, 159]. Lead poisoning is said to cause rickets [206].

Aids to Clinical Diagnosis of Rickets. In severe cases of rickets the diagnosis is simple, but in early cases doubt may be felt over the significance of mild beading of the ribs, dubiously enlarged epiphyses, late teething, delayed closure of the fontanelle, mild spasmophilia or convulsions with no obvious bone changes, recurrent attacks of bronchitis, weakness and disinclination to walk, and general poverty of well being and health. In all ailing children it is wise to remember that mild rickets may be the cause of many rather vague symptoms. Rickets is a disease of general metabolism.

Whenever there is hesitation over the clinical diagnosis of active rickets the serum phosphatase should be estimated, this definitely confirming or disproving the diagnosis since other generalized bone diseases and hepatitis which may increase the serum phosphatase are of academic rather than practical interest. The normal value in infants under two years is between 5 and 10 units [151], age and sex making no difference [205]. Values between 10 and 15 units are probably pathognomonic of rickets, while higher values certainly are. Corner's outstanding paper [204] should be read for a detailed study of the plasma phosphatase in infants and its relation to the various clinical signs of rickets, X-ray findings and the level of phosphorus and calcium in the blood. The microchemical method usually employed for estimating plasma phosphatase is described by King, Haslewood and Delory [208].

The blood calcium of infants is normally 10 to 11 mgm. per 100 c.c., and in the usual type of rickets is little if at all below this level. In the "low calcium" type of rickets associated with spasmophilia this calcium may be as low as 4 mgm. but is more often between 5 and 7 mgm.

The inorganic phosphorus is normally 4.5 to 5.5 mgm. per 100 c.c., which commonly falls in rickets to 2 to 4 mgm., 4 mgm. being generally taken as the dividing line between the normal and the rachitic. But a low level of either phosphorus or calcium is by itself no proof of rickets; when, however, the product of the two is below 40, this is definitely suggestive of active rickets.

The typical X-ray appearances of the bones in active and recently healed rickets have been described on p. 648. For the diagnosis of mild rickets, however, X-rays have several drawbacks. Generally the only rachitic changes in infants under one year of age are found in the skull and ribs, but the surrounding tissues prevent these being satisfactorily X-rayed. As a consequence the wrist has to be examined and changes here are uncommon before the end of the first year. Even after this age a negative

X-ray does not prove that rickets is not present. In a positive X-ray (Figs. 166 and 170) the distal end of the ulna shows "cupping" while in both it and the radius the smooth end of the diaphysis appears irregular and rough. The increased width of the cartilage is difficult to recognize with certainty, though spreading up along its sides the faint shadows of the slightly calcified perichondrium may be visible. The early changes in rickets must be diagnosed by experts, and even these are liable to differ in their opinions. Much confusion is caused by the ends of rapidly growing bones showing slight irregularities which suggest rickets though they are



FIG. 170. Active rickets in an English child. Note the enlargement and deformity of the epiphyses with lightly calcified masses of osteoid tissue in one wrist, and in the other the uneven ragged diaphyseal ends of the radius and ulna, with slight cupping of the latter. (See also Fig. 171.)

really normal, being due to calcification being so rapid that it is not quite uniform [91].

Differential Diagnosis of Rickets. In congenital syphilis the X-ray changes are moth-eaten epiphyses and periostitis while clinical stigmata are probably present. Of course rickets, being common in congenital syphilis, may complicate the radiological and clinical picture.

In osteogenesis imperfecta confusion will be avoided if the difference in the changes in the skull are remembered (p. 672) and the blue sclerotics. There is a frequent family history. The X-ray appearances are not those of rickets, and the blood calcium, phosphorus, and phosphatase are normal.

Chondro-osteo-dystrophy in older children will not be mistaken for rickets if an X-ray is taken, since the epiphyses are fragmented and the lumbar vertebræ are deformed.

Achondroplasia leads to an unduly long body compared to the short



FIG. 171. Healed rickets in the child shown in Fig. 170. The X-ray, taken five years later, is of the wrist which was most affected.

legs and arms: the hands are like star fish: the head is domed. X-rays and the usual biochemical investigations confirm the diagnosis.

Scurvy (p. 600) causes acute pain when the child is moved and there is bruising, bleeding gums if the teeth are erupted, red blood cells in the urine, and subperiosteal hæmorrhages and epiphyseal separations—the latter never occur in rickets, while pain is never severe and there is no

hæmorrhagic tendency. Rickets and scurvy can, of course, occur together.

Renal rickets and other forms of "rickets" are discussed on p. 681.

Treatment of Rickets. The amount of vitamin D required for the treatment of most infants with moderately severe rickets is about 1,200 I.U. a day. Individual needs vary greatly. The correct dose is that which cures the rickets. Cases are reported where as much as a million international units have been necessary [92], but when doses above 20,000 I.U. have to be given very careful biochemical and radiological control must be used to avoid the dangers of overdosage. As long as the serum calcium remains below 12 mgm. and there is no radiological proof of healing it is safe to increase the dose, though it must be remembered that toxicity may occur with a normal blood calcium (p. 648).

The various sources of vitamin D have been discussed on p. 652 so that here it is only necessary to point out that up to 1,200 I.U. daily can be given as cod liver oil, but that higher doses need the use of one of the concentrated fish liver oils.

Some physicians advise very large amounts of vitamin D, by that means hurrying the cure. There seems no advantage in most cases over a lower dosage and slower recovery. The only exception is in infants where the weakness and collapse of the ribs is threatening death from pulmonary infections [98]. Here doses of 50,000 units a day may be given in order to calcify the ribs with the greatest rapidity; X-ray and blood examinations must be used to check the results. If the infant is too marasmic to take a normal amount of milk, calcium phosphate gr. 10 should be given daily to supplement the mineral intake.

The use of artificial sunlight as an alternative or aid to cod liver oil has been discussed on p. 652.

Mineral salts given without vitamin D are valueless and calcium preparations containing neither magnesium [210] nor phosphorus [216, 217, 218] are definitely injurious.

Improvement is hard to judge clinically. The changes in the bones fade away too slowly to be of value in gauging the success of treatment, except where craniotabes is present, when healing should be obvious in two or three weeks. The general condition of the child furnishes some evidence. He is better in himself, happier, and his muscles appear stronger.

X-ray examination is a simple and generally available method of assessing treatment. After three weeks on average curative doses of vitamin D a faint line of preliminary calcification should appear running across the clear epiphyseal cartilage just beyond the end of the diaphysis. The further X-ray changes due to healing have been described on p. 648.

Phosphorus and calcium estimations are of value; within two weeks they should be approaching normal.

The blood phosphatase levels approach the normal more slowly than those of the minerals, falling in two to three weeks, but not becoming normal for about three months. A fall in the phosphatase is the best guide that healing has started [151, 209].

Treatment of Rachitic Deformities. Rest is the essential treatment

both for preventing and curing rachitic deformities, since only at rest is the weight of the body taken off the soft bending bones.

In mild cases carefully controlled exercise and insistence on regular rest after lunch is sufficient. Where deformities have already occurred splinting may be necessary to prevent the child crawling or walking until the bones have become recalcified. This is absolutely essential in girl babies, as their pelvis must be protected from distortion. General massage is invaluable when no exercise is taken; a masseuse can rapidly teach the mother how to give it.

The body's power of remoulding deformed bone is remarkable, but where the deformity is gross, surgical correction may be necessary. The suggestion that the bones should be deliberately decalcified to such an extent that they may be bent back to a normal position is bad.

The prognosis in deformities is excellent, except in the pelvis which in girls may cause serious trouble over childbirth in later life. Some abnormality of the chest is liable to persist, and deformities acquired at puberty when growth is nearly over will not disappear of themselves.

Treatment of Spasmophilia. The immediate domestic treatment of convulsions is to put the child in a hot bath at a temperature of 100–105° F.

Calcium deficiency being the immediate cause of spasmophilia the level in the blood should be raised rapidly. Calcium chloride gr. 15 thrice daily in milk may be tolerated and is ideal, since it is not only rapidly absorbed, but also, as the chloride ion is absorbed more than the calcium, it increases the ionization of the latter in the blood, by acting on the acid-base equilibrium. If calcium chloride is badly tolerated calcium lactate gr. 30 may be given instead. In continuous convulsions 5 c.c. of a ten per cent. solution of calcium lœvulate or gluconate should be given intravenously if possible, but if not into the buttock. Calcium chloride, 1 c.c. of a two per cent. solution, is excellent, but it causes ulceration unless it is given into a vein, and for this reason can only be used in an emergency when no other calcium preparation is available.

Sedatives should be given to all children who show any signs of spasmophilia. Vitamin D acts slowly and until it acts there is always the risk of general convulsions, which may even end fatally.

Bromides are safe. For an infant of six months 8 grains thrice daily should be given as a preventative, or three grains every two hours if the convulsions are continuous. The drawback is that some infants develop skin rashes. Phenazone in $\frac{1}{4}$ grain doses by mouth is an excellent alternative to bromides for children sensitive to the latter. Chloral causes gastritis if taken regularly by mouth, but it is valuable given in 2 grain doses per rectum. Chloroform may be necessary to check continuous convulsions.

Other Forms of Rickets. *Cœliac Rickets.* Cœliac rickets is only rickets complicating cœliac disease: a common complication because the inability to absorb fats reduces the amounts both of vitamin D and calcium acquired by the body. Treatment consists in giving calcium lactate in large doses by mouth and also vitamin D. Irradiation is ideal (see p. 652), but if it is too costly a highly concentrated fish liver oil, which will have little fat, must be used.

Scurvy-Rickets. This is a combination of scurvy and rickets.

Renal Rickets. This condition, also known as renal infantilism, renal dwarfism, renal osteitis fibrosa cystica and renal osteodystrophy [211], is due to damaged renal function preventing the excretion of phosphorus, with the result that it rises to such high levels in the blood that calcium ionization is depressed and little calcification can occur. Renal rickets is commonest in mid-childhood. It is characterized by rickets, dwarfism,

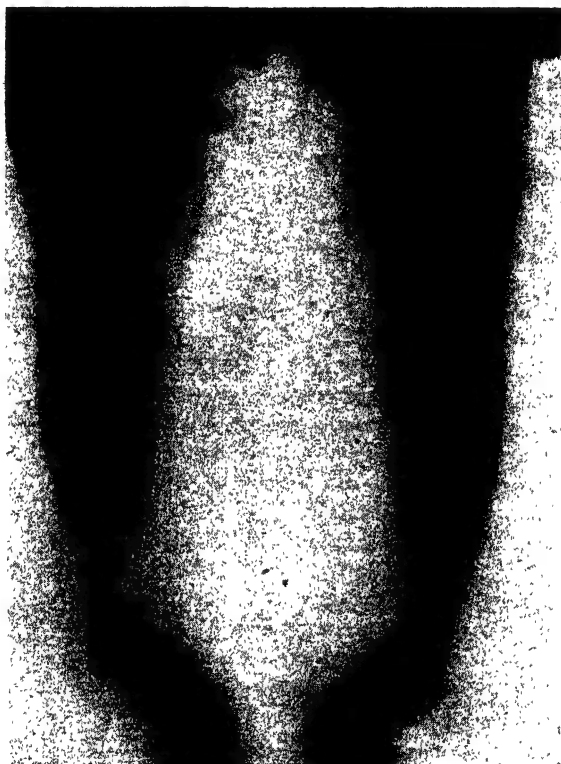


FIG. 172. Renal rickets in an English infant. Note the displacement of the distal tibial epiphyses, and the slight calcification of the expanded osteoid tissue.

albumin in the urine, a high blood phosphorus and impaired renal function. Vitamin D makes the condition worse by further mobilizing phosphorus from the tissues. A high calcium low phosphorus diet may delay the hopeless march of the disease by aiding the bowel in the excretion of the excessive phosphorus.

Rickets in Obscure Metabolic Derangements. From time to time cases of rickets which do not respond to vitamin D are reported [94, 95, 96, 97], the underlying cause being some obscure metabolic upset. Cases of the "Fanconi Syndrome" generally show dwarfism, severe osteoporosis more

than rickets, a low blood phosphorus and renal glycosuria; cystinuria from faulty cystine metabolism has also been reported in this group [98].

OSTEOMALACIA (MOLLITIES OSSIIUM)

Osteomalacia of pregnancy, hunger osteomalacia, and probably many cases of senile osteoporosis are fundamentally all the same, being due to a deficiency of vitamin D. In the past they were not recognized as identical because the obvious cause appeared to be repeated pregnancies, famine, or old age rather than a common vitamin lack. They are, indeed,

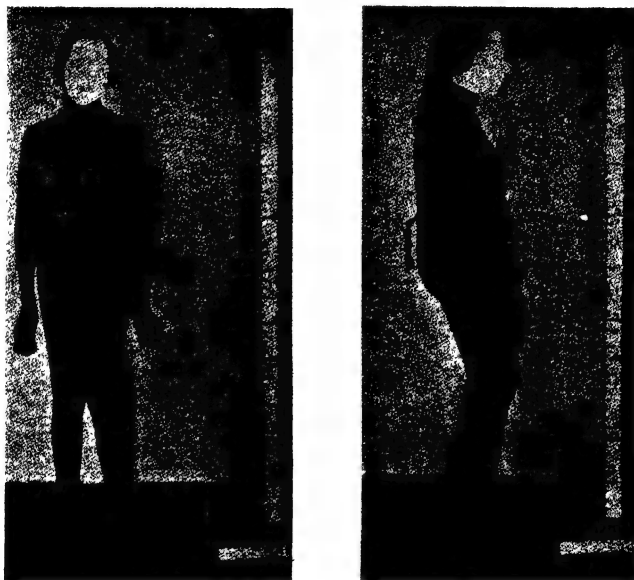


FIG. 173. Osteomalacia in a Sikh woman. The condition, due apparently to poverty and pregnancy, started in 1939 while living in England. Note the absence of the normal lumbar lordosis, the adduction of the thighs, the left genu valgum, and the abnormal height of the fundus at the third month of gestation. (See also Figs. 174 and 175.)

the adult equivalent of low calcium rickets modified by the absence of growth.

Osteomalacia is such a dramatic disease that sufferers have taken their place in myths and legends almost as if they were giants. The French of the last century besides making the best clinical study of the condition [4] have collected many historical accounts [99, 100, 101]. The best known was a dwarf who in the sixth century lived to be three hundred years old, but appears to have been more like a jelly fish than a man so soft were his bones. He could not move, and was plagued by the teasing of dogs and children: his compensation was his fame, since he could be twisted into any position at will. Then there was a woman called Supiot who attracted much attention in 1700, and another nameless, who "Ayant été d'une taille très élevées, devint, avant de mourir plus petit qu'un

nain." Trousseau's account [4] of Madame Rehbin, however, is still the only perfect description of the disease, and the perfect example of possibly the greatest physician's mastery of prose.

Osteomalacia of Pregnancy. This is now a rare disease in Western civilization, though cases are still occasionally seen [174, 245], and MacLennan [245] suggests that mild osteomalacia in England may be the reason why some women who have had no difficulty in earlier labours may appear in later ones to have a slight contraction of their pelves. When osteomalacia was more common it was generally among young women who had had frequent pregnancies on bad food and no sunlight. But it was also found during first pregnancies, and then the mother was often over thirty. With each child the condition takes a step forward as the body is further drained of its minerals and vitamins. And with each child the condition becomes more piteous: the mother is more imprisoned in the house by pain and deformities; her chances of earning more money, of gaining more food, more sunlight, are curtailed; her pelvis collapses further and further, making her next confinement even worse than the last. Sometimes her downward progress halts between her pregnancies, sometimes she may even improve if fortunately she does not suckle her infant in the vain hope of thus warding off her tragic fertility.

The gradual extinction over five hundred years of the Norse colony founded by Eric the Red in Greenland at the end of the tenth century was apparently due to the pelvic deformities of osteomalacia hindering childbirth. It is thought that the Norse colonists did not eat the local diet of fish and fish oils which is necessary to supplement the scant sunlight in Arctic regions [102].

Hunger osteomalacia. This is found quite apart from pregnancy during periods of chronic famine. Both sexes suffer and the elderly more frequently than the young, who are more exposed to the sun. The condition is endemic in areas where the diet is poor, or custom keeps women indoors, such as some parts of China and India. There is a seasonal variation, fewer cases being seen in the sunny part of the year when sunlight helps to eke out the deficient diet. Rarely hunger osteomalacia is found in large cities where solitary old people live in proud self-respecting poverty rather than apply for charity. We have recently seen such an old woman, who could only hobble along with difficulty and had lived for years alone in London.

Senile Osteoporosis. This appears to be often a mild form of hunger osteomalacia, and not, as was formerly held, a senile atrophy of bone [85].

Symptoms, Diagnosis, and Treatment of Osteomalacia. The earliest symptom in osteomalacia is a vague tenderness or aching pain in the bones of the lumbo-sacral region and hips, which is worse when the patient walks. It is generally called "rheumatism." As the condition progresses the tenderness and aching of the bones spreads so that percussing the chest may cause the woman to cry out. She may even refuse to be touched at all and wince when approached.

Bony deformities often occur with surprising rapidity. The woman appears to shrink. She loses height partly by the bowing of her legs, chiefly by the softening of her spine, which may become twisted and always

bows forward, so that the ribs pile up over one another like the spokes of a fan, even coming to rest on her pelvis. The pelvis itself becomes grossly distorted until childbirth, from being difficult, becomes impossible. Trousseau [4] found a woman for whom a sound had to be used instead of digital examination, the pelvic outlet was so small. The legs twist and bend in any direction. Generally in the old it is the spine, in the young the pelvis and legs which are most affected (Figs. 173 to 176).

Fractures from trivial falls, from the normal pull of the muscles, or

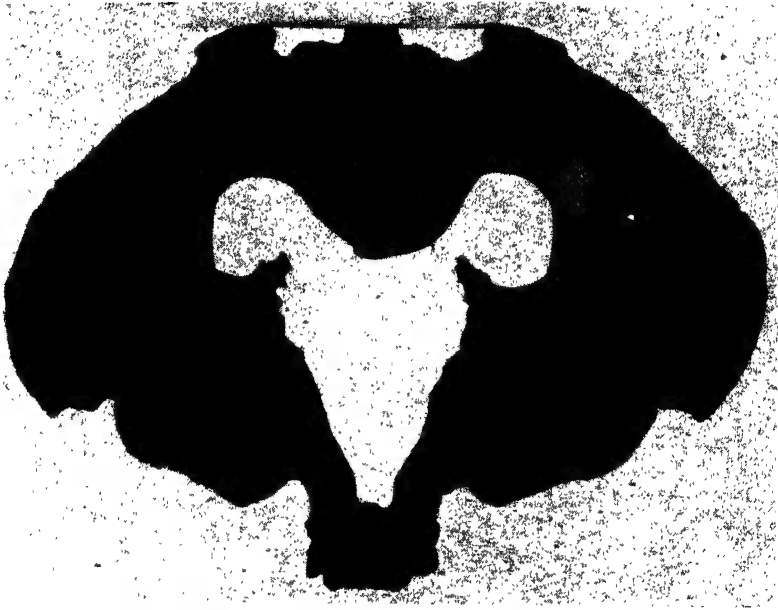


Fig. 174. Osteomalacia. The Shansi pelvis presented by Professor Maxwell to the Royal College of Surgeons. Note the diminished size of the pelvic outlet, which makes childbirth impossible (see also Figs. 173, 174 and 175).

from no apparent cause, are common. They heal slowly or not at all, false joints forming instead.

Muscular weakness and flaccidity analogous to that of rickets occurs; on getting up the patient climbs up herself like a child with muscular dystrophy. The adductor muscles of the thighs tend to go into spasm due to the aching of the bones (Fig. 173). The walk becomes a wide based waddle compounded of weakness, pain, and deformity.

Cataract, according to Maxwell and Pi [174], is found in at least fifteen per cent. of marked cases. The condition develops slowly, and in its early stages can only be diagnosed with a slit lamp. It is important to consider whether cataract in young and middle-aged women may not be caused by undiagnosed osteomalacia which is still only causing vague pain in the back and limbs. Treatment of the underlying deficiency of vitamin D may improve the cataract.

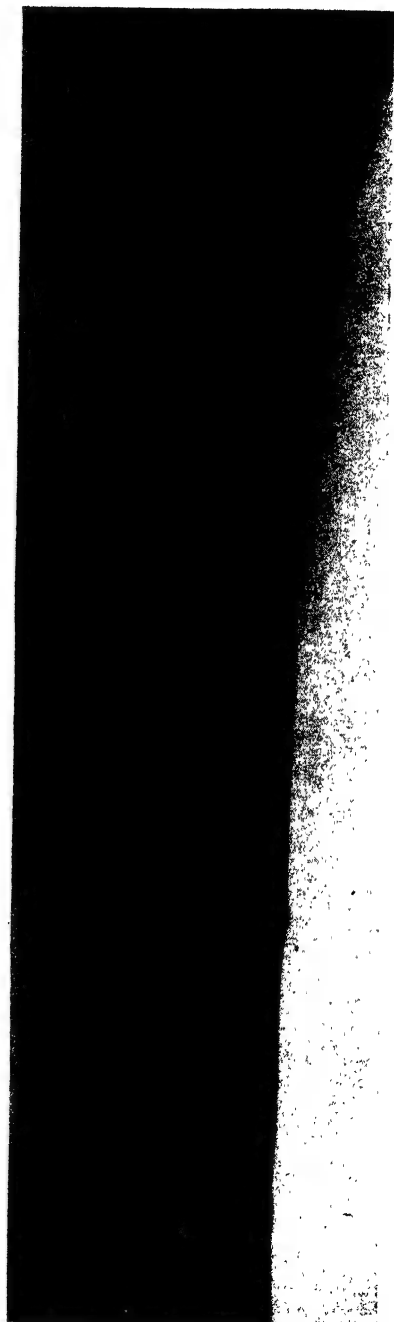
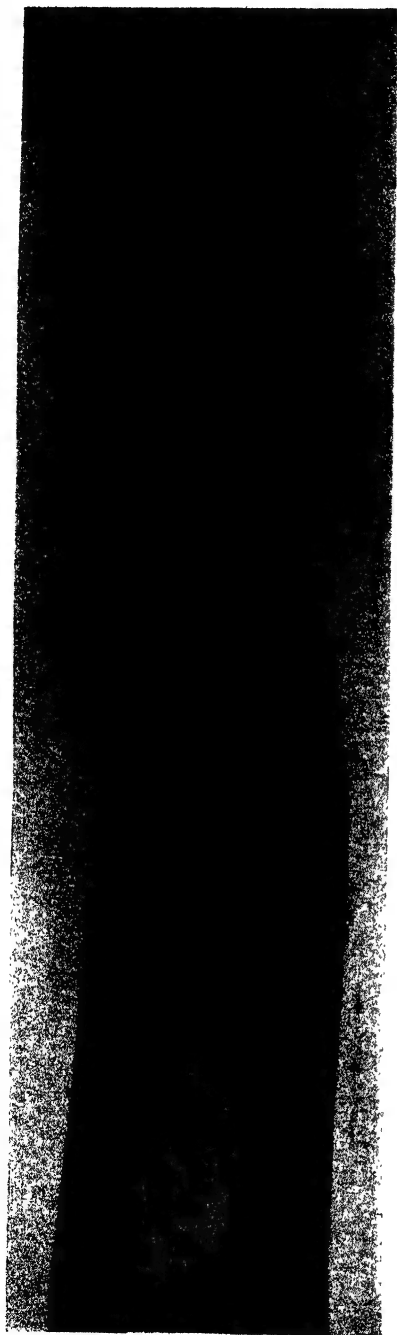


FIG. 175.

English Osteomalacia.

FIG. 176.

X-ray of bones of forearm : (*right*) showing Looser's zone ; (*left*) control normal forearm.

Tetany is common, as would be expected from the low blood calcium [174]. The parathyroids may be increased in size, probably in an effort to increase the low blood calcium.

The teeth are not affected. Taylor and Day [178] studied twenty-two Indian women with severe osteomalacia and found only thirty-four cavities in five hundred and sixty-five teeth. Eight of the women had no dental decay whatsoever. These observations are of great importance as confirmation that dental caries is not due to a deficiency of vitamin D (p. 689).

Menstruation and fertility in women are not, unfortunately, affected. The ovaries have been said to contain an abnormal number of follicles.

In all forms of osteomalacia the blood calcium is generally low, often to tetanic levels. The inorganic phosphorus may be normal or low [174], while the phosphatase is slightly increased. The startlingly light bones, which are often so soft they may be cut with a knife or bent like cardboard, largely consist of osteoid tissue; their enlarged cavity is filled with hæmorrhagic osteoid tissue or fat; their ash contains little calcium, though the phosphorus is, by comparison, high. Magnesium is increased. The urine on standing forms a phosphatic scum.

The diagnosis rests on the dietetic history, the tenderness, weakness and deformities, X-ray examinations, the levels of the serum calcium, phosphorus, and phosphatase, and the response to treatment. Sometimes the pain in the ribs is so acute that this, combined with the bronchitis which is so common, suggests pleurisy; a suggestion which is only discarded when the pain is found to be acute all over the ribs, which not only are exquisitely tender but in their weakness crepitate beneath the light pressure of the hand. The generalized rarefaction of the bones shown by radiography will differentiate the disease from any unlikely confusion with Paget's disease or generalized osteitis fibrosa cystica.

Death, unless treatment is given, is from pulmonary infections and the toxæmia from bed sores.

The outlook as regards life is excellent with treatment; some recovery of stature and amelioration of the deformities may be hoped for.

Treatment is with large doses of vitamin D. Probably about 8,000 I.U. daily will be enough, but much larger doses may be required. To supply the necessary minerals for the recalcification of the bone calcium phosphate gr. 80 thrice daily should be given, as well as plenty of milk, cheese and fish. Radiography and calcium estimations should, if possible, be used to make certain adequate vitamin D and calcium and phosphorus are being given.

Extra calcium may have to be given if tetany persists (see p. 681) or if treatment causes the blood calcium to sink further because it is diverted to the bones, thus bringing on tetany.

In the past ovariectomy has been strongly advocated [99]. It is quite unnecessary and unjustifiable.

DENTAL DECAY

There is such a widespread belief that lack of vitamin D is the chief cause of dental decay that it seems important to consider the subject in

some detail. Dental decay, or dental caries, is due to the destruction of the enamel and dentine of the teeth by saprophytic organisms. Their growth and the progress of decay may be assisted by any one of several different factors. The more important are (a) the use of refined foods, especially white flour and white sugar, (b) the use of heated milk instead of raw milk, (c) the failure of the individual to produce "immune saliva," and (d) possibly, but improbably, a diet deficient in vitamin D.

Refined Foods. The bad effects of refined foods have been observed for many years. The most classical observations were made on the island of Tristan de Cunha. When the island was visited in 1982 caries was practically non-existent, especially among people of under fifty. No food was imported, the islanders living entirely on the unrefined produce of the island. In the next five years, however, a large amount of refined flour and sugar was left by visiting ships, so that the inhabitants suddenly had added to their diet foods which they had never had before save in minute quantities at long intervals. The result from the point of view of dental decay was tragic. Barnes [108] observed that in only five years caries from being almost unknown in children was common, while in adults between forty and fifty years old it had increased by fifty per cent.

If further proof is required of the effect of refined foods it is furnished by the teeth of the Bantu [104]. These people had exceptionally fine teeth, but when they started eating refined European foods caries became as common as in England. In one generation perfect teeth, which have often been considered to be an inherited racial characteristic, were utterly lost. That it was the fault of their new foods appears certain because there was no other change in their manner of life which appeared to have any possible significance. The increase in dental decay in the island of Lewis also tells the same story [105].

It is generally stated that white flour and sugar cause decay by clinging round the teeth while coarse fibre-containing flours and sugarless foods being less sticky are easily removed by the natural self-cleansing action of the mouth—the "detergent diet" of Sim Wallace. The sticky white flour and sugar are decomposed by bacteria, thus producing acids which erode the enamel. This appears to be true, but is not the whole truth about the bad effects of refined foods.

The work of Osborn and Noriskin [106] showed that unrefined sugars and cereals have a protective action against the destruction of enamel. When they incubated human teeth with saliva and white flour, or refined sugar, the enamel was dissolved. If, however, wholemeal flour or unrefined sugar were used the enamel was hardly affected, especially with the unrefined sugar.

The protective substance present in unrefined food has not yet been isolated, but its action is not dependent on any change of acidity in the saliva. It explains the puzzling observations that dental decay may be absent in the mouths of native children whose teeth are always coated in sticky sugar from eating raw sugar cane all day [104].

Heated Milk. Raw milk, that is unpasteurized and unboiled milk, has a very marked protective action against dental decay. This was found to be so by Sprawson [107], who noticed a sudden startling fall in caries in

a children's institution. The only change which had been made was giving each child daily a pint of raw milk. Further enquiries among dentists confirmed that children brought up on raw milk—either cow's or goat's—were free of caries. Sprawson [108] also points out that in the island of Pitcairn caries was very common, while in Tristan de Cunha it was very rare. The only difference between the diets was that in the latter island raw milk was drunk though probably less vitamin D was taken.

In the first edition of this book the contentious subject of pasteurization was discussed in its wider aspects. As, however, one of the authors (F. B.) believes pasteurization is unwise while the other (F. P.) believes in its value, it seems better to omit this wider discussion, referring the reader to the sections in each chapter for the narrower issue of how pasteurization alters the individual vitamins of milk.

Immune Saliva. Fish [112] has pointed out that some human saliva prevents decay and some does not. Dog's saliva always does so, while monkey's saliva varies as in man. When Fish placed carious human teeth in a dog's mouth in a few days they were sterile; the saprophytic organisms which cause decay had been destroyed. The same occurred if a carious tooth was incubated with human saliva from a man with no active caries. But saliva from a mouth with progressive caries did not sterilize the tooth nor did that from a monkey with caries. There is, therefore, a quality in immune saliva which protects teeth from the organisms of decay. This immune saliva is most frequent after adolescence and is commoner in men than women [113]. If it is the product of a healthy general metabolism it would explain why a good diet gives protection against caries in children, without having to postulate the unsatisfactory theory that the protection apparently given in some cases by vitamin D is directly due to improved dental calcification [188].

Traces of fluorine in the drinking water cause mottling of the enamel of the teeth. Such teeth are very resistant to decay, even when the mottling is so slight as not to be aesthetically objectionable [181]. It is possible that the fluorine, excreted into the saliva, acts by inhibiting the growth of saprophytic organisms in the mouth: in other words a form of immune saliva is produced.

Vitamin D. In rachitic children the teeth tend to erupt late and decay early. The late eruption appears to be a direct effect of too little vitamin D, since Speidel and Stearns [163] report that there is an optimum intake of vitamin D above or below which eruption is delayed. The early decay is probably due to the refined carbohydrate diet such children generally eat. The teeth of rachitic children, may, however, be perfect. Both Taylor and Day [114] and Wilson [175] have reported many Indian children with severe active rickets who had no caries, even when born of osteomalacic mothers. Their diet was very coarse; this coarseness appeared to be the reason for the absence of decay.

In osteomalacia, as has been pointed out on p. 687, the teeth do not decay unduly though here the lack of vitamin D and calcium is profound.

Vitamin D is therefore not *necessary* for good teeth in either the foetus or the child or the adult. But the question arises as to whether vitamin D

is *valuable* in helping to protect the teeth. To give the answer before the evidence: the addition of extra vitamin D to the diet appears in some cases to decrease dental decay, but we believe this to be due to the improvement in general health brought about by removing a mild deficiency of vitamin D.

The evidence that vitamin D is valuable for the teeth is based firstly on animal experiments and secondly on human investigations. The animal experiments were chiefly done by Lady Mellanby on dogs [115]. She showed that the teeth of rachitic puppies had poor thin enamel and the dentine was what she called hypoplastic. These teeth did not decay, since dogs have a naturally immune saliva (p. 689). One also wonders whether part of their poor structure was due to the puppies never chewing or biting anything hard. "Function creates structure," and in these puppies the teeth had no function—the food was pap, they were too ill from their rickets to gnaw their cages as did the healthy control puppies, and when they were allowed outdoors they were muzzled.

However, having shown that rachitic puppies have poor teeth which do not decay, the next step was to investigate the shed milk teeth of children which had decayed. Those teeth which had most caries were found to have the poorest structure, reminiscent of the rachitic puppies' teeth. This was held to show that the teeth decayed because they were bad, and bad because they were rachitic. This argument only contains two possible fallacies: firstly, bad teeth may be associated with decay because chronic bad health—from whatever cause—might hinder both the proper formation of the teeth and the production of immune saliva; secondly, there was no reason to believe the children who had bad teeth were rachitic [116] except that they had bad teeth—in fact the argument having gone in a circle gets nowhere.

The clinical value of vitamin D was tested by giving it as a supplement with groups of children and comparing their teeth to those of children who had had no such supplement. Many investigations [116] both on a large and a small scale in England and America showed that by increasing the amount of vitamin D in the diet not only was the number of teeth which subsequently developed caries reduced, but that caries which had started tended to be arrested. It must, however, be noted that full protection was not given. Thus in one investigation over a period of about eight months the children receiving "abundant fat soluble vitamins" had on an average 1.4 teeth showing fresh or increased caries, against 5.1 teeth in children who had little vitamin and some oatmeal. So that though the teeth were far better preserved with extra vitamin D, yet the degree of caries was still deplorably high. McBeath and Zucker [117] found the natural vitamin more valuable than the synthetic.

The arguments against vitamin D being directly responsible for this improvement are:—

(a) Some investigators have not found that vitamin D has any effect, even on recently erupted teeth, when given for over a year to large groups of children [117].

(b) Fish [118] found experimentally that there was no evidence that vitamin D had any effect on formed dentine or enamel, so that it is difficult

to understand how the enamel, and so the resistance to decay, could be improved by vitamin D.

(c) The faults in the rachitic teeth of puppie; are not like the faults where caries starts in human teeth [118].

(d) The teeth which should show most hypoplasia, if this is due to lack of vitamin D, are those which are formed during the first year and a half of life when rickets is most common. The incisors should thus be affected most; they are affected least. Then the most affected in order are the second molars, first molars, canines, though they erupt in the order of first molars, canines, second molars [119, 120]. Thus there is no relation between hypoplasia and the teeth formed at the period when vitamin D tends to be most deficient.

(e) Children with extensive progressive caries do not commonly show any signs of rickets [116], and children with severe rickets, even when born of osteomalacic mothers, may have no caries (p. 689).

(f) Dental caries in rats is not prevented by vitamin D [158].

We are, then, left with the fact that vitamin D decreases caries to some extent in some children, but that caries is not dependent on a deficiency acting directly on the teeth themselves. It appears most likely that vitamin D improves caries only indirectly by improving the general health and nutrition of the child—both factors which have an effect on caries [114, 121, 188].

Immunity to dental decay depends on eating coarse unrefined food—especially unrefined sugar and stone ground flour—raw milk and a good diet. There is no simple specific against decay such as vitamin D, and cleaning the teeth cannot compensate for a bad diet.

The absolute necessity for vitamin A at ages when the enamel is being formed, and the importance of vitamins A and C for the health of the gums, and so indirectly for the preservation or rather fixation of the teeth, must be remembered.

Dental Decay and Pregnancy. The old saying that "every child costs a tooth" appears to be only explicable on the spread of paradental disease during pregnancy. Actual dental decay during pregnancy does not appear to increase even under the added strain of osteomalacia (p. 687). Fish [124] states: "There is no active evidence as yet of any definite loss of calcium from the dentine under any circumstances," including pregnancy, low calcium diets, excessive vitamin D, and parathormone injections. Protection during pregnancy depends on the dietetic factors already mentioned, on cleaning the teeth, and not simply on taking more vitamin D.

FURTHER CLINICAL USES OF VITAMIN D

Endocrine Disorders. Cretinism. Cretins when they start taking thyroid often grow amazingly fast. Rickets as a result may occur, so the possibility must be kept in mind and ample cod liver oil given as a prophylactic measure.

Thyrototoxicosis. The action of vitamin D in very large doses on the thyroid has been discussed on p. 646. The possible value of cod liver oil, as far as its vitamins are concerned, is probably due to its vitamin A.

Parathyroprivic Tetany. The use of vitamin D in controlling the tetany due to damage of the parathyroid glands is most valuable, since it raises the low blood calcium and decreases the high blood phosphorus. It also stabilizes the calcium and phosphorus metabolism so that there is less tendency to sudden tetany [45], but as the effect of vitamin D in relieving tetany takes several days, it is useless in post-operative emergencies. In prolonged treatment it has the great advantage of not losing its effect, while parathormone, on the other hand, does so after repeated administration.

There is the danger that large doses of vitamin D may have toxic effects, but by using the natural concentrated fish liver preparations, by keeping the dose at the lowest level and stopping it for short periods where possible, harmful effects are unlikely to occur (p. 696).

The dose of vitamin D must depend entirely on the individual case. With a diet high in calcium and low in phosphorus 20,000 units should be given daily by mouth and the amount adjusted from the clinical results. Sevringhaus and St. John [286], however, state that the diet need not be carefully controlled since they have successfully treated six women who ate as much meat, milk and eggs as they liked. Four of the women were observed for over two years and one of them had an uneventful pregnancy. Vitamin D, combined with calcium salts, was given orally in doses of 150,000 to 400,000 I.U. daily, the correct amount being adjusted partly by blood calcium estimations and partly by subjective symptoms, of which tingling in the arms and hands was the earliest and most reliable indication for increasing the dose.

Dihydrotachysterol, one of the products of the prolonged irradiation and hydrogenation of ergosterol called commercially A.T.10 (anti-tetanic preparation number 10) has been used instead of vitamin D for the treatment of tetany by Snapper [125], Himsworth and Maizels [172], and many others. It has no appreciable antirachitic effect [201], but raises the blood calcium more rapidly than vitamin D. It is, however, more toxic, and in some cases has to be given in increasing amounts. It also has a more erratic effect on the level of the calcium in the blood. It appears to have no advantages and severe drawbacks compared to vitamin D, apart from its more rapid action in raising the blood calcium.

Sexual Disorders. An occasional increase in libido of both sexes and also in sexual capacity, and an improvement in the rhythm of the menses, has been reported in a small proportion of cases who were taking very large doses of vitamin D [45]. The therapeutic use of vitamin D for sexual disorders, however, is ruled out by the dangerously high amounts which must be given, and by the small proportion of patients who will benefit.

The Bleeding Tendency in Jaundice and Hepatic Insufficiency. It is very surprising that vitamin D is seldom used for preventing the hæmorrhage which is so common and so disastrous in patients with jaundice, or hepatic insufficiency, who have to undergo an operation. In a very large series of carefully controlled cases Gray and Ivy [178], and McNealy and others [179] found that when the "Ivy" bleeding time was prolonged the operative outlook was bad because of hæmorrhage during or after the operation. But vitamin D given thrice daily by mouth, in doses of about

7,000 I.U. for four to fourteen days before operation, almost invariably reduced the bleeding time to normal, and prevented any hæmorrhagic complications. When there was no bile in the stools bile was given by mouth to aid the absorption of the vitamin. Where the hepatic insufficiency was very profound vitamin D did not improve the bleeding time, nor did it decrease a hæmorrhagic tendency in any condition not associated with liver damage. Why vitamin D only has an effect in one kind of hæmorrhage is obscure: it does not act through changing the blood calcium [178], or through correcting the prothrombin deficiency [200] which is often found in jaundice owing to poor absorption of vitamin K (see also pp. 647, and 797).

Lead and Radium Poisoning. Lead behaves like calcium from the point of view of storage in the body [86, 60, 126, 158]. During the acute stages of lead poisoning when it is important to decrease the ionization of the lead in the blood, and to remove it rapidly from the circulation, 800 I.U. of vitamin D a day should be given to aid the deposition of the lead in the bones. Calcium lactate, 180 grains daily, should be given at the same time, both to decrease the ionization of the lead and also to ensure that lead and calcium are being deposited and not mobilized in the bones.

After the acute symptoms of lead poisoning are over it is necessary to "de-lead" the bones. Vitamin D in large doses is not so certain in its results as parathormone, and both need careful supervision. Ammonium chloride must be used when patients cannot be carefully guarded against excessive mobilization of lead bringing back the acute symptoms of poisoning. In any case the diet should give a low calcium high phosphorus intake—the latter to depress the ionization of the lead in the blood [86, 60].

Radium is so toxic that it must be eliminated from the body as quickly as possible, vitamin D being of less value than the other measures mentioned for "de-leading" [60].

The Healing of Fractures. About 600 I.U. daily of vitamin D should be given to patients with fractures. This ensures that the calcification of the callus shall not be prevented by lack of vitamin D which is especially important in the old [85, 101] where mild osteoporosis is common. Massive doses of vitamin D delay calcification [234]. A.T. 10 (p. 792) has also been extensively, and to our mind unwisely, used to hasten calcification. Irradiation has also been suggested [127]. The use of vitamin C in the healing of fractures is discussed on p. 602.

Arthritis. Reed and his collaborators [45] sum up the effect of using vitamin D in arthritis thus: "A group of forty-five cases of atrophic arthritis, seven cases of hypertrophic arthritis and three mixed cases were treated with 150,000–200,000 units of vitamin D daily with definite measurable improvement in 74·5 per cent. of cases as manifested by reduced swelling and pain, increased mobility (active and passive), improved muscular tonus and weight gain." The authors point out that there is a definite risk from such large doses, though apparently less than one per cent. of cases showed toxic symptoms. Small doses should be given at first to avoid risk in unduly susceptible patients (p. 696). Treatment has to be continued for many months or even years. No claim is made for any

physiological reason for this form of treatment. Good results have also been reported by other workers [140, 141, 238]. But Freyberg [228] from a most careful study, in which he used the reputedly pure "activated" vitamin D, reports that six out of thirty-six patients had to give up treatment because of toxic symptoms and that "this form of therapy should not be relied on as the only form of treatment, for seldom is the course of the disease favourably altered." The general impression is left that other forms of treatment over such a long period would have as good results without the very real danger of toxic damage.

Skin Diseases. In psoriasis Maynard [157] and Brunsting [129] have reported complete success in many cases, though not all. Treatment had to be continued for many months, and the doses given were 800,000 to 500,000 I.U. a day, which of course may be toxic. Madden [176], however, states that in twenty-four patients "the degree of improvement in the psoriasis corresponded to the severity of the reaction to vitamin D. When the reactions subsided the psoriasis recurred." Thacker [190] finds cod liver oil more effective than calciferol. Krafka and Wright [189] have reviewed the subject, and the latter from his own observations on forty-five patients considers treatment with vitamin D no better than any other treatment. In the most thorough investigation yet made, which was reported by Clarke [189] and was carried out by twenty-two doctors on one hundred and forty-one patients, only twelve per cent. improved.

Scleroderma [180, 157] has also been improved on doses of 200,000 to 800,000 I.U. a day. Improvement is reported to be slow, starting after about four months and not being finished in fourteen. The reason advanced for the use of vitamin D is that in scleroderma there is a retention of calcium and phosphorus, and that calcium is laid down in the collagen fibres of the corium—possibly from chronic infection.

Acne often improves or is cured on doses of 5,000 to 6,000 I.U. daily [181, 157]. Leukonychia totalis and pemphigus [157] and also eczema and epidermolysis bullosa [189] have all been treated with vitamin D, but no beneficial results have been satisfactorily confirmed.

Ulceration of the skin and burns have been widely treated with local applications of vitamin D ointments and cod liver oil preparations. Reports vary as to their value. In our own experience cod liver oil and vitamin D ointments were quite ineffective in diabetic ulcers, bed sores, and varicose ulcers. Whatever effect the oil may have is probably not due to its vitamins [191]: the subject is discussed more fully on p. 791. Chilblains have been cured by a multiplicity of treatments. The important factors appear to be comfort and good food—especially butter and eggs, which have been claimed to act by their vitamins. Ultra-violet light is also valuable.

Dementia Præcox and Narcolepsy. Vitamin D was given by Notkin and others [182] to thirty-two patients with dementia præcox, but the results were probably quite negative. Fessler [183] reported a good effect in two cases of narcolepsy. Since neither rickets nor osteomalacia affect the mentality the use of vitamin D in mental disorders is most unlikely to be effective, unless the use of toxic doses is employed for some obscure "shock therapy."

Neurological Diseases. Cod liver oil injections have been claimed by Leak [187] to aid in the recovery of the central nervous system in subacute combined degeneration and of vision in disseminated sclerosis. These effects have not been confirmed by anyone else.

Asthma and Hay Fever. Rappaport and Reed [184] in 1938 stated that raising the level of the blood calcium with vitamin D has an excellent effect in patients sensitive to pollens. The dose was high—100,000 to 300,000 I.U. daily—and as a means of raising the blood calcium vitamin D is unreliable (p. 648). Presumably lack of success has been the reason for this form of treatment not having been used recently.

Ophthalmic Conditions. In myopia [185] and trachoma [186] no results have been obtained with vitamin D given by mouth, while in iritis and conjunctivitis [187] injections of vitamins A and D are said to be of value, and so is cod liver oil applied to the eye in phlyctenular conjunctivitis [188]. The improvement in the latter two conditions was probably caused by vitamin A (p. 36). Keratoconus [156] has been reported to improve with calcium and vitamin D. There is no confirmation of the experimental and clinical work of Knapp [249] that lack of vitamin D causes retinitis pigmentosa in the dog and that in man this condition, myopia and night blindness are improved by vitamin D and calcium.

General Infections and Tuberculosis. The lack of experimental evidence in animals that vitamin D has any effect on raising resistance to infections apart, possibly, from poliomyelitis, is discussed on p. 647. But relieving the body of the general strain of a possible mild vitamin A and D deficiency is of value, and so warrants the use of cod liver oil as a general dietetic measure in infections.

It should be noted that neither rickets nor osteomalacia are associated with sepsis—except catarrhal infections probably due to other causes (p. 671). So it seems improbable that lack of vitamin D has any relation to sepsis. Leak [187], however, over a period of many years in general practice has found injections up to 5 c.c. of a sterile oil containing 60,000 I.U. of vitamin A and 10,000 I.U. of vitamin D per cubic centimetre has a remarkable effect on general infections—a result he implies is due to the vitamin A (p. 55). Stanley-Jones [189] also states that intramuscular injections of vitamins A and D are excellent for localized septic infections.

Spiesman [188] believes that vitamins A and D given orally reinforce each other in affording protection against colds.

Measles has been treated with vitamin D with no effect [144].

An unimpressive and unconfirmed Austrian report [248] states that about 40,000 I.U. daily given by injection, combined with calcium and salicylates by mouth, is of value in rheumatic endocarditis in children: there is a latent period of two weeks before improvement occurs.

Tuberculosis for years has been treated with cod liver oil [74]; it seems probable that the other constituents of the oil rather than vitamin D explain any good effect (p. 792). It is an old observation that rachitic children seldom are scrofulous, and women with osteomalacia do not get consumption [101]. While lack of growth in the scrofulous child explains the absence of rickets, the absence of consumption in osteomalacia does not. Vitamin D is important in giving protection against tuberculous

infections. The calcifying action of vitamin D in toxic doses on the soft tissues cannot be used for increasing calcification in pulmonary lesions, because the other soft tissues of the body get calcified at the same time.

VITAMIN D POISONING

All preparations of synthetic vitamin D [238]—however pure they are reputed to be [228]—are toxic to man in large doses and there is no reason to suppose that the vitamin of concentrated fish liver oils is not toxic, though no human cases of poisoning have been reported apart from two children who died both from drinking large amounts of cod liver oil and from prolonged sun bathing.

The dose of vitamin D which, when repeated daily, is toxic, varies greatly with the individual. Cases have been reported where only 25,000 I.U. daily of a very pure preparation of "activated" vitamin D have caused toxic symptoms [228], though a review of the clinical results of many workers [238] shows that most patients tolerate doses of from 200,000 to 400,000 I.U. daily. Reports seldom mention how soon toxic symptoms may appear, but with very large doses—700,000 I.U.—they have been reported within ten days. However, Steck and his collaborators [43, 45] state, from extensive observations on over seven hundred patients, that few show toxic symptoms unless the dose is over 10,000 I.U. daily per pound of body weight. Though the experience of these investigators is very extensive and though their statements on vitamin D are generally accepted without cavil, yet in this instance it would appear that the doses they say are safe are far too high. The safety of single massive doses of vitamin D is discussed on p. 661.

Vitamin D is an extremely powerful drug. Poisoning has been widely reported, even being fatal, so that the use of large doses of vitamin D is only justified if the patient is under constant supervision. The danger of poisoning increases with the commercial provision of more concentrated preparations with no accompanying warning of their possible toxicity.

The laboratory work on poisoning with vitamin D has been described on pp. 647, 649, where it was pointed out : (1) that poisoning could occur with a normal blood calcium ; (2) that metastatic calcification of the soft tissues depends to some extent on excessive calcium in the diet ; (3) that the artificial irradiated preparations of vitamin D were more toxic than the natural forms ; (4) that the brunt of the poisoning fell on the kidneys and large blood vessels, and that disease of the former decreased the tolerance for the vitamin.

From the clinical point of view it would appear, therefore : (1) that if large doses must be given it is useless to rely entirely on blood calcium estimations as a safeguard, the clinical symptoms of poisoning being a better guide ; (2) that the giving of highly concentrated vitamin D and calcium preparations together is unwise ; (3) that fish liver oils should be the source of high doses of vitamin D ; (4) that in patients with nephritis or cardiovascular disease large doses are unwise.

Indications for Using Large Doses of Vitamin D with Caution. Fat patients, as their fat is largely inert from the point of view of metabolism,

should not be given very large doses to begin with. Patients confined in bed may also metabolize vitamin D slowly, since two with fractures rapidly developed serious toxic symptoms and the healing of the fractures was delayed [284].

Personal sensitiveness is important and can be avoided only by beginning with small doses. One patient has been reported who showed toxic symptoms on 25,000 I.U. daily [228] and one infant, who was frequently outdoors in the sun, died from taking a concentrated cod liver oil which only gave him about 1,500 I.U. daily [68].

Nephritis and cardiovascular degeneration are contraindications for the use of high doses of vitamin D, since two elderly men with such conditions have been reported by Steck and others [48] as dying from poisoning with vitamin D.

Indigestion and constipation, according to Reed and his collaborators [45], often bring on symptoms of poisoning in patients who, till then, have tolerated their vitamin D perfectly.

Emotional strain even may cause toxic symptoms to appear [45].

Children require watching. One child we saw in hospital died from both being exposed to the sun, and also drinking not only his own cod liver oil but also that of several other children in his ward.

Symptoms of Poisoning [16, 43, 44, 45, 68, 128, 134, 228, 238]. General well being and a good appetite is often the first symptom of poisoning. In this lies a danger for doctors who buy large bottles of concentrated irradiated products for dispensing and drink them, often in huge doses, for the tonic effect that they give.

Loss of appetite follows quickly on the sense of well being, and loss of weight greater than would be expected from the loss of appetite. After this intestinal symptoms such as nausea, vomiting and diarrhoea increase rapidly.

The urine is increased and voided frequently by night and day. It is loaded with calcium and phosphorus and contains calcium casts if it is not too acid [92]. Shortly before death dehydration occurs with scanty urine. The blood pressure is not affected.

Weariness and weakness, and more rarely profound mental depression come on, and may be early symptoms. The memory occasionally is confused.

Headaches are usual, and one special form has been noticed which may be the first symptom to arouse suspicion. This is a tightness across the back of the head which goes on to acute sensitiveness of the scalp, so that the patient cannot rest his head on the pillow. The same tenderness at the back of the head has been found in dogs. Pain along the jaws and tender teeth have been noticed, and pains in joints and muscles. Profuse sweating may occur. The hands and feet occasionally feel numb and tingle.

Aphrodisiac effects to the extent of social inconvenience have been occasionally noted without other toxic symptoms.

In the infant reported by Thatcher [68] the symptoms were loss of appetite and weight, and on examination a few days before he died he was a pale thin feeble infant, with small and very soft muscles; he was moderately dehydrated; his urine was scanty and contained a little

albumin; his cerebrospinal fluid was normal in spite of several convulsions before he died.

Ross and Williams [68] have described four infants poisoned by vitamin D, of whom one died. They were only taking thirty drops of a commercial preparation of calciferol three times a day, but this dose provided 20,000 to 40,000 I.U. of vitamin D. Such a fatal overdose, of course, would have been impossible had ordinary cod liver oil been prescribed. X-rays of all the infants showed increased density of the zone of provisional calcification, with an area of rarefaction proximal to this. There was also periosteal thickening with a dense outer layer and a rarefied inner zone. In the infant who died the post-mortem showed calcification of the arteries, cardiac muscle, kidneys, bronchi, alveoli, and stomach.

Treatment. Stopping the vitamin D generally relieves the symptoms in a few days, but in severe cases, especially in infants, intravenous normal saline should be given, since the cause of death is the loss from the body of its electrolytes [142, 176]. This loss is due to the diuresis caused by the kidneys' efforts to secrete the excessive calcium and phosphorus. When the toxic symptoms are very mild they may be controlled by vitamin B₁ or yeast [45, 238], thus allowing treatment to be continued.

Prognosis. The outlook is good. As has been said on p. 648, the damage to the tissues and even the calcification disappears in animals. In man there is no evidence as to the ultimate effect of prolonged overdosage. No bad chronic effects have been noted in patients who have had toxic symptoms, apart from two in whom there was a persistent inability to secrete a concentrated urine though all other tests of renal function were normal [234]. The post-mortem examination of Thatcher's infant [68] showed calcification of the kidneys, but apparently not to such a degree that good functional recovery should not have been possible. Thomasen's boy [148], who had taken magnesium, calcium, and irradiated ergosterol almost continuously for four years showed, radiographically, calcification of his large arteries; his later history is not known.

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CHAPTER VIII

VITAMIN E

THE ANTISTERILITY OR ANTIDYSTROPHIC VITAMIN. ALPHA, BETA OR GAMMA-TOCOPHEROL

VITAMIN E is the name generally used when speaking of the vitamin in general or as it occurs in foods. Alpha-tocopherol is the most biologically active of several very similar substances, all of which have the properties of vitamin E. Thus while alpha-tocopherol means one distinct substance, vitamin E may mean either alpha-tocopherol, or a mixture of this and other similar substances. To avoid confusion vitamin E should be only used in the latter sense, and not as a synonym for alpha-tocopherol. The name tocopherol is derived from the Greek *τόκος* childbirth, and *φέρω* to bear.

HISTORY

Herbert McLean Evans, of California, will always have his name associated with vitamin E, partly because he and Bishop [1] in 1922 demonstrated the existence of an antisterility vitamin and partly because of the monograph on vitamin E written by himself and Burr [2] in 1927. This work remains to the present day the foundation of our knowledge of vitamin E. The authors showed that the foods richest in the vitamin were green leaves and the germ of seeds. Wheat germ and wheat germ oil were found to have a remarkably high content of vitamin E, and remain to this day the best source. Rats were the experimental animals used, and for these animals it was proved that a deficiency of vitamin E leads to sterility in the male and abortion, though not failure to conceive, in the female.

The existence of the vitamin, however, had been foreshadowed in 1920 by Mattill and Conklin and confirmed in 1922 by Mattill and independently in 1923 by Sure. This early work is summarized by Evans and Burr [2].

Until 1928 vitamin E was thought to be entirely concerned with reproduction, but in this year Evans and Burr [3] reported that young rats suckled by vitamin E deficient mothers became paralysed, while Goettsch and Pappenheimer [4] in 1931 showed that guinea-pigs and rabbits when deprived of vitamin E developed a primary muscular dystrophy histologically identical with the progressive muscular dystrophies of man. From Denmark Ringsted [5], in 1935, and Einarson and Ringstød [6], in 1938, published careful and extensive research on the effects of lack of vitamin E on the central nervous system of adult rats. They pointed out that the neurological degenerations which were produced resembled those of amyotrophic lateral sclerosis and tabes dorsalis in man.

It is a depressing demonstration of the lack of co-ordination between research workers and clinicians that it was not until nine years after the discovery of vitamin E that Vogt-Møller [7] in Denmark first put it to any useful purpose by treating sterility in cows. In the same year he treated

two women with habitual abortion with success [8], and six years later Young [18] in England, and Shute [14] in Canada, reported good results in the treatment of threatened abortion and pregnancy toxæmias.

Bicknell [9] in 1938, seven years after the possible value of vitamin E in human muscular dystrophy had been implied by animal research, started to treat cases of muscular dystrophy and neurological degeneration with vitamin E, or, rather, wheat germ. The improvement in his cases was reported in 1940. Stone [10] and Wechsler [11] in 1940 reported cases of muscular dystrophy and amyotrophic lateral sclerosis successfully treated with vitamin E, but it was only in 1941 that the subject began to arouse wide clinical interest. The early promise, however, of the value of vitamin E in the treatment of muscular and nervous diseases has not been confirmed by later work which, at best, only suggests that these diseases are caused by some complicated failure in the metabolism of the vitamin which can only be corrected in rare cases by simple treatment with the vitamin alone.

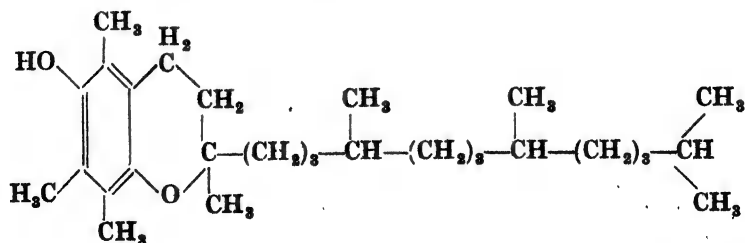
The elucidation of the chemical structure of vitamin E has been rapid since 1936. In this year Evans, Emerson and Emerson [12] isolated from wheat germ oil two alcohols, alpha- and beta-tocopherols, and from cotton seed oil gamma-tocopherol, all of which had vitamin E activity.

Further work by many investigators including Todd, Bergel and Drummond and their collaborators in England, Fernholz in America, and Karrer and John on the Continent finally led to the synthesis of alpha-, beta- and gamma-tocopherols and the elucidation of their chemical structure [7].

CHEMISTRY OF VITAMIN E

The naturally occurring substances which are most effective in preventing sterility in rats are alpha, beta and gamma-tocopherol, and there are also various synthetic compounds which possess some slight activity [7, 16, 165]. The chemistry of the tocopherols is extremely complex; the papers by Todd [7], Karrer and Bergel [7] and Evans [16] and Rosenberg's book [195] should be consulted for discussions on the subject.

The formula of alpha-tocopherol is



Beta- and gamma-tocopherols are isomers, possessing one less methyl group than alpha-tocopherol in the aromatic nucleus.

The biological activity of the tocopherols is partly dependent on the constitution of the side chain, and partly on the substituents of the aromatic nucleus. Joffe and Harris [196] report that for preventing sterility in rats the relative potencies of natural alpha-, beta- and gamma-

tocopherols are 100, 40 and 8.8, while Gottlieb and his collaborators [197], comparing the synthetic racemic tocopherols to each other, give relative values of 100, 25 and 19.

Synthetic racemic alpha-tocopherol and its esters have only about two-thirds of the biological activity of the naturally occurring dextrorotatory forms, while the difference between the synthetic racemic and the natural dextrorotatory beta- and gamma-tocopherols is even greater [196]. Different esters vary greatly [195, 196], the allophanates for instance being inert while the acetate, which is the ester generally used in clinical medicine, is as active as the alcohol [197]. The relative potency of the tocopherols for preventing muscular dystrophy is not known. Alpha-tocopherol, at body temperatures, has the greatest antioxidant potency, though at higher temperatures gamma-tocopherol is more active [251].

Vitamin E as it occurs in wheat germ oil or in vegetable and animal tissues is remarkably stable. It is an important biological antioxidant for fats and is soluble in all lipid solvents though not in water. Heating wheat germ for three hours in air at a temperature of 170° C. does not destroy the vitamin, nor does distillation of wheat germ oil *in vacuo* at 250° C., nor aeration at 97° C. Cooking of plant or animal tissues is not harmful, but prolonged exposure of wheat germ oil to strong ultra-violet light leads to some destruction of vitamin E. It is comparatively stable to saponification with strong alkali and to strong hydrochloric acid [2]. Pure tocopherols are unstable in air, and when exposed to ultra-violet light, but the esters are stable [7]. Crystalline alpha- and gamma-tocopherols have been prepared, though at room temperatures the tocopherols are oils giving maximum absorption at 290–294 millimicrons. For a further account of the physical properties of the tocopherols and their esters the papers by Robeson and his colleagues [198] should be consulted. Rosenberg [195] reviews the methods by which the tocopherols are synthesized or extracted from biological materials.

Rancid fats rapidly destroy vitamin E, a point of great importance discussed on p. 729.

ESTIMATION OF VITAMIN E

All the earlier work on the estimation of vitamin E in plant and animal tissues was handicapped by there being no stable preparation of pure vitamin E itself which could be used as a yard stick or unit, so that it is only since about 1940 that accurate assays of vitamin E have been carried out. Even now the laborious biological technique has to be employed for accurate estimations as other methods [251] for differentiating between the highly active alpha-tocopherol and the relatively inert beta- and gamma-tocopherols have not yet been fully investigated.

There are three methods of estimating vitamin E: biological, chemical, and spectroscopic.

Biological Estimation. The foundations of the biological method were laid by Evans and Burr [2], the criterion of vitamin E activity used being the cure or prevention of the particular kind of sterility brought

about by deprivation of vitamin E (p. 719). These authors pointed out that the only certain method of knowing that a doe on a vitamin E deficient diet was really depleted of vitamin E was for her to have a typical resorption pregnancy. Only then was such a doe fit to be given the dietetic supplements whose vitamin E content was being examined. It was necessary to mate her with a buck of proved fertility and to make certain that both positive mating and implantation had occurred. Then if a litter was born it was good proof that the dietetic supplement contained vitamin E. Time could not be saved by omitting the preliminary absorption gestation, because the animals varied so much in their initial

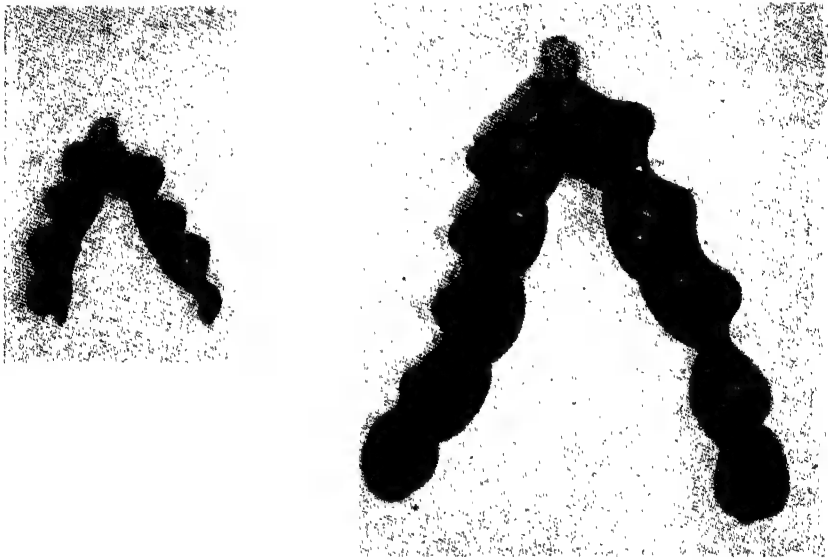


FIG. 177. The uterus on the left is from a pregnant rat on a normal diet, while that on the right is from a pregnant rat on a diet deficient in vitamin E. Note the smaller number of embryos within the latter, and their arrested development.

stores of vitamin E that even in litter mates, kept on the same deficient diet, some rats might show "first litter fertility," and some might not.

Bacharach [17], however, has perfected a technique by which the preliminary absorption gestation can be omitted, with great saving of time. He stresses that there are "very large and unpredictable variations in the average response of groups of animals at different periods even though the pre-experimental and experimental conditions have, as far as possible, been kept constant." He found, for instance, during one series of experiments that the mean fertility dose of a substance appeared to increase threefold [7]. This emphasizes the importance of using a stable standard preparation as a control during all estimations. Bacharach [15] has also worked out a dosage-response curve for vitamin E which shows that in comparing two substances for their vitamin E activity

Gottlieb, Quackenbush and Steenbock [197] have found in rats that, with a diet low in fat, the increase in weight during pregnancy is, within limits, in direct proportion to the amount of vitamin E supplied. This method of assay is economical, since the animals can be used again and few are needed because of the graded nature of the response. It is also claimed to be more sensitive than other methods, enabling amounts of vitamin E to be determined which are too small for the production of litters.

The antidystrophic activity of vitamin E may also prove of use in biological estimations. Mackenzie and McCollum [18] report that the creatine excretion in the urine of rabbits rendered dystrophic through lack of vitamin E decreases rapidly when vitamin E is added to the diet (p. 728). Even if this cannot be used in quantitative estimations it should have value in qualitative ones, being less arduous and quicker than fertility investigations.

Dam and Glavind [19] have suggested that the alimentary exudative diathesis of chickens reared on vitamin E free diets might be employed in biological estimations of vitamin E (p. 784).

Chemical Estimation. There are three chemical methods in use for estimating tocopherols; those of Karrer and Keller [20], Emmerie and Engel [7, 21], and Furter and Meyer [22]. The first two methods are based respectively on the reduction by tocopherols of gold chloride and ferric chloride. The amount of the reduction of the ferric chloride is followed colorimetrically, while that of the gold chloride by more complicated electrometric methods. According to Smith and Bailey [7] both methods are equally accurate, and both suffer interference by other reducing substances, especially the carotenoids which are often present in vitamin E concentrates. Parker and McFarlane [23] and Hines and Mattill [201] have suggested modifications for removing carotenoids and other substances which interfere with the reduction of the ferric chloride, the latter workers describing a technique for estimating vitamin E in liver and muscle which is probably accurate to within ± 8 micrograms. Other workers have adapted this method for estimating vitamin E in the blood [200], obtaining good agreement with biological assays.

Until 1944 chemical estimations could not differentiate between alpha-

tocopherol and the other relatively biologically inert tocopherols, so that chemical estimations of vitamin E in foods made before 1944 tend to give too high values from a biological point of view. Hove and Hove [251], however, in 1944 described a chemical method by which alpha-tocopherol can be estimated separately from the other tocopherols.

The nitric acid method of Furter and Meyer [22] depends on the colorimetric measurement of the red colour given by tocopherols with nitric acid. Smith and Bailey [7] and Emmerie and Engel [7] agree that this is of little value, since the colour reaction is also given by the oxidation products of the tocopherols, which are themselves biologically inactive but may occur in old vitamin E oils.

Spectroscopic Estimation. Vitamin E gives maximum absorption at 290–294 millimicrons. Cuthbertson, Ridgway, and Drummond [24] have reported that spectroscopic methods give good results when estimating vitamin E in animal tissues. With their technique as little as 0.01 per cent. of a tocopherol can be detected in the unsaponifiable fraction of animal fats, and estimated with reasonable accuracy when it is above 0.08 per cent. Vitamin A does not interfere with the estimation.

UNITS OF VITAMIN E

The international unit of vitamin E is the amount which, when administered orally, has the same specific activity as 1 mg. of synthetic racemic alpha-tocopheryl acetate in preventing resorption-gestation in rats deprived of vitamin E.

PHYSIOLOGY OF VITAMIN E

Nothing is known of how plants synthesize vitamin E, though it is possible its origin is closely related to that of vitamin K [165]. All animals so far investigated appear to be dependent on plant tissues for the vitamin, not forming it themselves.

Absorption. There is little evidence on how vitamin E is absorbed. On analogy with the other fat soluble vitamins A, D and K, fat in the diet and a normal secretion of bile should help absorption. The possible importance of fat should not be overlooked now that concentrated or synthetic fat free forms of vitamin E are widely used. On the other hand, rancid fats rapidly destroy vitamin E (p. 729).

Bile appears to be necessary for the absorption of vitamin E. Brinkhouse and Warner [27] report that dogs with chronic biliary fistulae develop within seven to nine months both the nutritional muscular dystrophy and testicular degeneration typical of a deficiency of vitamin E (pp. 722, 726). In one dog the muscular dystrophy was arrested and improved when bile was fed by mouth. Greaves and Schmidt [27] have obtained similar results in rats.

Liquid paraffin probably hinders the absorption of vitamin E [28] as it does that of vitamins A, D and K.

Some vitamin E escapes absorption and appears in the faeces of rats when the diet is very rich in the vitamin [24, 201]. The amount lost may be a quarter of that eaten, males excreting more than females [24].

The foetus absorbs vitamin E from the mother as long as she has adequate stores or receives it in her diet [2]. If the mother is deficient in vitamin E placental transfer is impaired, since Pappenheimer [29] has reported rabbits on a vitamin E deficient diet giving birth to dystrophic young. Absorption through the placenta must be insufficient to allow for storage, since the young born of rats and rabbits on a deficient diet develop muscular dystrophy only slightly sooner than do the young born of normal animals when both are suckled by a vitamin E deficient doe [3, 80]. The tissues from newly born rats of mothers on a normal diet contain only small amounts of vitamin E [2].

The amount of vitamin E in milk depends on the maternal diet [3, 80]. Pappenheimer and others [219] report that half of a single dose of 51 mg. of alpha-tocopherol was excreted in the milk of nursing rats during the whole period of lactation.

Whether vitamin E undergoes any change in the intestine during absorption is not clear. Many workers, among them Evans and Burr [2] and Knowlton and others [86], have reported that vitamin E is active when injected for the prevention and cure of both sterility and muscular dystrophy. In the latter condition, however, Mattill [87] and others [155, 156] found alpha-tocopherol more active when given by mouth than by injection. Wechsler and his collaborators [157] report that the level of vitamin E in the blood of three patients actually fell after intramuscular injections of 100 mg. of alpha-tocopherol, though the level rose when the vitamin was given by mouth.

Storage. The foetus or suckling animal, as was seen in the last section, has at best low stores of vitamin E. Adult animals can store vitamin E for a considerable period, Evans and Burr [2] finding that a single dose of vitamin E might suffice the rat for three or even four normal pregnancies. Biological assays by Mason [189] on the relative amounts of vitamin E in various tissues of the adult rat have shown that the liver stores large amounts when the intake of natural mixed tocopherols is high and less than any other tissue when the intake is low: storage is never as great as that of vitamins A and D. Active mammary tissue has the highest content of all organs, and the heart, lungs and spleen are twice as rich in the vitamin as the other viscera, muscles and body fat.

Chemical assays by Hines and Mattill [201], which they suggest fail to estimate all the vitamin in the tissues, do not agree with Mason's findings since in rats, whatever their intake, the liver always contained large amounts of vitamin E compared to the muscles. The values—expressed as milligrams of tocopherol per kilogram of tissue—in animals receiving (1) a vitamin E concentrate equivalent to 100 mg. of tocopherol daily, (2) a "normal" diet which probably contained very little vitamin E, and (3) a vitamin E deficient diet, were for liver and for muscle 42.3 and 11.9; 22.1 and 7.5; 22.6 and 4.8. For rabbits the values were 86.8 and 28.1; 9.2 and 8.0; 9.4 and 5.7.

Lundberg and others [202], basing their assays on the effect of alpha-tocopherol on the induction period (p. 717) of abdominal fat, found that storage in the fat of rats did not reach a maximum until seven to ten days after a single dose of 50 mg., after which the amount stored fell to half its

maximum in two months. With an intake of 50 mg. daily for ten days the maximum amount stored was 97 mg. per kilogram.

Cuthbertson and others [24], using a spectroscopic technique, also found the greatest storage in body fat and some storage in muscle, but none in the liver, heart, kidneys, adrenals, testes, ovaries and pituitary. The failure to find any vitamin E in so many organs is presumably due to the technique being less delicate than the biological and chemical techniques used by the other workers mentioned above.

The contradictions in these various reports about the relative amounts of vitamin E stored in different tissues are difficult to explain, but it may be that different mixtures of the tocopherols are not stored in the same manner, and that the rates of absorption by and distribution from the various tissues differ, so that storage depends both on what forms of tocopherol have been given and how soon afterwards the tissues are examined. Hines and Matill [201] have suggested that the high and very slowly depleted hepatic stores of the rat may explain why this animal is more resistant to lack of the vitamin than the rabbit whose hepatic stores are low, especially compared to those in the muscles.

The level of vitamin E in the blood of animals [17, 24, 205], varies from 0.29 to 1.1 mg. per 100 c.c. In man the level in adults is 0.59 to 1.62 mg., with an average of 0.96 mg. [157] to 1.20 mg. [200], and in children 0.72 to 1.12 mg. [203]. The level in children with muscular dystrophy is said to be normal [203], though Wechsler and others [157] found the average in fourteen cases of "myopathies" was 0.61 mg. In one child with celiac disease [203] it was 0.64 mg. The level in adults may rise with massive oral doses—740 mg. daily—to 2.0 mg. and with a single oral dose of 1,500 mg. Quaife and Harris [200] report that the level in one subject rose to 2.60 mg. in six hours, then very slowly falling, being still 1.92 mg. at the end of forty-eight hours. With an oral dose of only 300 mg. the maximum level is also reached in six hours, but it is not so high and the return to normal occurs in twenty-four hours [255]. Intramuscular doses depress the level [157] which is analogous to the effect of injecting vitamin A.

Varangot [250] in France in 1942 reported values at the beginning of pregnancy of 1.25 mg. per 100 c.c. which rose throughout pregnancy to 1.82 mg. at term and then rapidly fell to normal. These figures are extremely interesting because in 1943—that is, after many further months of the German occupation and starvation of France—he reported that the average value in fifteen non-pregnant women was 0.22 mg. and in twenty-one men 0.19 mg. In Holland also unusually low levels have been reported during the German occupation [255]. Vitamin E has not been demonstrated in the cerebrospinal fluid [255].

Destruction and Excretion. The depletion of stores of vitamin E was shown by Evans and Burr [2] to continue at the same rate whether rats became pregnant or not, though vitamin E is excreted in milk (p. 789). It thus appears that vitamin E is used in the normal processes of the body, pregnancy not appreciably increasing the consumption. When large amounts of vitamin E are taken they are rapidly destroyed; where this occurs is obscure [24], though it may be in the muscles, partly because

vitamin E plays an important part in muscular metabolism (p. 717) and partly because Pappenheimer and others (p. 788) have shown that the vitamin is only essential for the integrity of working muscle, and therefore is possibly metabolized and destroyed during muscular work.

It is improbable that the first stage in the destruction of vitamin E is its oxidation to tocopherylhydroquinone, which is biologically inert [84], though this has been suggested because the blood of dogs contains 0.46 mg. per 100 c.c. and that of man 0.81 mg. per 100 c.c. [205]. Hines and Mattill [201], however, found none in the liver, muscles and urine of rats consuming 100 mg. of vitamin E daily, which is a strong argument against vitamin E being oxidized to this substance.

Succinylsulphathiazole is reported, from well-controlled work on young rats [206], to accelerate the development of muscular dystrophy in vitamin E deficient animals. Sulphaguanadine, however, has no such effect. In man vitamin E has been reported as preventing neurological symptoms during treatment with sulphathiazole (see pp. 768 and 773).

Excretion of vitamin E in the urine and faeces of rats only occurs when it has been consumed in very large amounts [24, 201]. It has not been found in human urine [255].

Requirements. Different species vary greatly in their requirements and in the effects which a deficiency produces (p. 718). In the rabbit, in which muscular dystrophy is the dominant effect of a deficiency (p. 726), requirements depend on body weight—but not on age or sex [156]—being, according to Mackenzie and McCollum [18], from 0.7 to 1.0 mg. daily of alpha-tocopherol per kilo. of body weight. Much smaller amounts allow of growth over a long period and ward off muscular dystrophy, so that such mild dystrophic lesions as do occur give no clinical symptoms [82]. Eppstein and Morgulis [156] state that doses as low as 0.82 mg. daily per kilo. of body weight are sufficient for all needs.

In the rat, in which loss of fertility is the dominant symptom of a deficiency (p. 719), the male requires more than the female. Evans and Emerson [187] gave rats of both sexes from weaning 0.1, 0.25 and 0.75 mg. of alpha-tocopheryl acetate daily. The smallest dose protected both sexes from muscular dystrophy and enabled the females to have three litters, though ultimately there was uterine discoloration (p. 720) and the suckling young all developed muscular dystrophy. Daily doses of 0.25 mg. had a slightly better effect, but even doses of 0.75 mg. did not completely prevent dystrophy in the young of the third pregnancy. In the males doses of 0.75 mg. preserved fertility for at least sixteen months, while doses of 0.25 mg. and 0.1 mg. preserved it for nine and five months. Post-pubertal testicular degeneration is considerably delayed, but not prevented, by a single dose of 0.5 to 5 mg. when this is given on the fifteenth day of life; when given two weeks later or one week earlier, there is little or no effect [218]. Similar protection is provided by giving the nursing mother 51 mg. [219]. Gottlieb and others [197] give the total requirements for a normal pregnancy with normal offspring as 0.85 to 1.5 mg. when the diet contains no fat, and double this when the diet is rich in fat.

The male mouse does not require vitamin E for fertility, but the female does [188]. Protein appears to decrease the need for vitamin E,

since the dystrophy of deficient young mice is largely prevented by a diet rich in protein [220], which recalls the way in which vitamin E prolongs the life of rats on a diet containing fatally low amounts of protein [221].

Ducklings [39] require 2 to 4 mg. daily to prevent dystrophy, while the needs of the chick largely depend on other components of the diet (p. 788).

Human requirements are discussed on p. 748.

Fundamenta lor Biochemical Action of Vitamin E. The fundamental or biochemical action of vitamin E is probably to inhibit or restrain oxidation in all the tissues of the body. It must be admitted that this view of its action does not explain why some tissues are more affected by lack of the vitamin than others, nor why different tissues are affected in different species unless it is postulated, which would seem reasonable, that some tissues, differing from species to species, have a prior claim on whatever amounts of vitamin E are available or can utilize anti-oxidants other than vitamin E.

Vitamin E *in vitro* is an anti-oxidant, and in the discussion on its relationship to vitamin A (p. 85), it was seen that probably this anti-oxidant activity protects vitamin A and carotene against oxidation not only while they are still within the lumen of the gut but also after their absorption when they are circulating in the blood. In a similar way vitamin E protects biotin and the vitamin B complex against destruction by rancid fats [237]. Vitamin E is the only anti-oxidant in body fats [207] and it plays the same rôle in at least some fish liver oils [208].

In the young rat deprivation of vitamin E causes a marked increase in oxygen consumption before there are any signs of muscular dystrophy [209]. When muscular dystrophy appears the oxygen consumption falls in rats [209] and remains normal in guinea-pigs [65], but probably this is only because partial inanition counteracts the effect of lack of vitamin E. The increase in oxygen consumption in the intact animal can be wholly accounted for by the increased oxygen consumption of the dystrophic muscles [74, 209, 210] and so does not necessarily involve an increase in any other tissues, which in the liver at least does not occur [209]. Injections of alpha-tocopheryl phosphate reduce the oxygen consumption of muscles, removed at biopsy, almost to normal within four hours, though the same effect is only achieved by oral doses of alpha-tocopherol in twenty-seven hours. The ester, but not the alcohol, also causes the same result in isolated slices of dystrophic muscle and reduces the raised activity of succinoxidase [210]. In normal children vitamin E decreases the basal metabolic rate and the specific dynamic action of glycine [164].

Phosphorus turnover in all the tissues in the body is stimulated by lack of vitamin E. This is thought by Weissberger and Harris [211] not to be a direct effect of the deficiency but a secondary result of the increased oxidation in muscle; since here oxidation and phosphorylation are coupled so that what affects one will affect the other. It is considered that this increased turnover in muscle leads to the increased turnover which occurs in both sexes equally in the bone, kidney, blood, uterus, ovary, testicle and muscle [211]. It is of profound interest that no greater change in phosphorus metabolism occurs in dystrophic muscle than in bone or blood,

and also that the change in the genital tissues—which in the rat are the first to be damaged by a deficiency—is no greater than that in other tissues. The ability to phosphorylate glycogen is decreased by forty per cent. in dystrophic muscle [73]. Vitamin E raises the blood sugar curve in normal children [164]. Kepinov's work on the effect of vitamin E on glycogen metabolism is discussed on p. 725.

Doses of vitamin E of at least one hundred times the normal requirements also cause an increase in the turnover of phosphorus, but this is not similar to that caused by a deficiency since it is greater, it occurs more rapidly and it is never associated with pathological changes in muscle, though, on the other hand, osseous rarefaction occurs [211].

The various phosphorus compounds and electrolytes in dystrophic muscle are present in abnormal proportions [73, 212], but these are not helpful in understanding the true nature of the metabolic disaster, since by the time a muscle has become dystrophic its analysis only reveals the sum of the compounds which occur in an uneven mixture of normal, degenerating, necrotic and calcified muscle fibres, wandering cells and connective tissue.

Creatine is decreased in dystrophic muscle [36, 67, 72, 210] and is excreted in large amounts in the urine even before there are any clinical signs of a deficiency of vitamin E [18]. Again this abnormal creatine metabolism may be held to be a secondary effect of the uncontrolled and excessive oxygen and phosphorus metabolism of the muscle. Alpha-tocopheryl phosphate injections reduce still further the creatine content of biopsy specimens of dystrophic muscle for two hours, after which time it slowly rises, presumably because the muscle's thirst for creatine has been slaked. Hottinger [164] states that giving 12–18 mg. of alpha-tocopherol daily to healthy children immediately decreases their normal creatinuria and also that caused by glycine, though that caused by creatine is not affected.

The other changes which occur as a result of a lack of vitamin E are a decrease in cholinesterase activity in the liver, brain, serum [213] and muscle [216], an increase in the cholesterol content of dystrophic muscles and the blood [212] and brain [153, 215]—though this has been denied for chicks [214]—and some increase in other lipoids in the muscles [215], but not in the heart or other organs [212]. It is interesting to note that alpha-tocopherol is said to raise the level of cholesterol and fatty acids in the blood of schizophrenics [191].

EFFECTS OF VITAMIN E DEFICIENCY

There is a remarkable variation in the response of different species to a deficiency of vitamin E. In the rat [2] the earliest sign of a deficiency is sterility in the male and failure in the female to carry pregnancy to term. If the deficiency is prolonged changes occur in many organs showing that vitamin E is not solely concerned with reproduction, but with most activities of the body. The endocrine system and kidneys may be affected (pp. 722, 735) and the central nervous system degenerates (p. 731). There is also a primary degeneration or dystrophy of voluntary and involuntary muscle (p. 726). In the mouse the female requires

vitamin E for reproduction, but the male does not. Muscular dystrophy, accompanied by œdema, occurs only in the first month of life and is not serious or progressive. Smooth and cardiac muscle, and the osseous and nervous systems are not affected [188]. In the guinea-pig dystrophy of voluntary muscles dominates the picture [4], though failure to carry pregnancy to term [161] and testicular degeneration [222] can occur in animals rendered dystrophic but not killed by diets only partially deficient in vitamin E.

A similar dystrophy of voluntary muscle is the earliest and as yet the only recognized symptom of deprivation of vitamin E in the rabbit [18, 160], hamster [210], sheep and goat [212], tree kangaroo [29] and duck [39]; in the turkey [40] it is the smooth muscle of the gizzard which is affected. The chick on a diet deficient in vitamin E develops encephalomalacia or an œdematous condition of the body (p. 788) and possibly muscular dystrophy [188]. The development of the embryo in the egg is arrested [42, 48] and the testes of cockerels degenerate [42].

The dog [44] develops muscular dystrophy, and probably a testicular degeneration [27], and there is some evidence that foxes also require the vitamin [45]. The cow and the pig appear to need the vitamin for reproduction [7], and Mr. D. I. Witherington informs us that vitamin E increases the fertility of mares and the stamina of race horses. Cats [46] are said not to require vitamin E, but this may be due to the deprivation not having been sufficiently prolonged to produce symptoms [44]. The occurrence of vitamin E in the queen bee's royal jelly [47] has not been confirmed [48].

In man a deficiency of vitamin E possibly causes abortion and muscular dystrophy, but whether or no the nervous system is affected is still obscure (pp. 743, 747, 766).

From all these different manifestations of a deficiency of vitamin E it can be seen that the name "antisterility vitamin" is due purely to the chance use of rats in the first investigations. Had guinea-pigs, for instance, been first used the name "antidystrophic vitamin" would have been as widely adopted.

It must be remembered that though synthetic alpha-tocopherol has been shown to prevent all the symptoms of a deficiency of vitamin E, yet there is no proof that other tocopherols may not be more potent in the cure of conditions apart from sterility; or that different species do not utilize beta or gamma-tocopherol better than alpha-tocopherol. It is indeed possible that the tocopherols mutually enhance each other's effects, and so should be taken together [228].

In the following pages vitamin E will be considered in its relation to (a) Reproduction; (b) The Endocrine Glands; (c) Growth; (d) Muscular Dystrophy and Rancid Fat; (e) Nervous Degenerations; (f) Nutrition of Chicks; (g) Other Tissues, Neoplasms, Resistance to Infection; (h) Other Vitamins.

The Relation of Vitamin E to Reproduction. Rats are the animals which have been chiefly used in studying the part played by vitamin E in reproduction. The results of a deficiency of vitamin E in the female and male are so different that they need to be discussed separately.

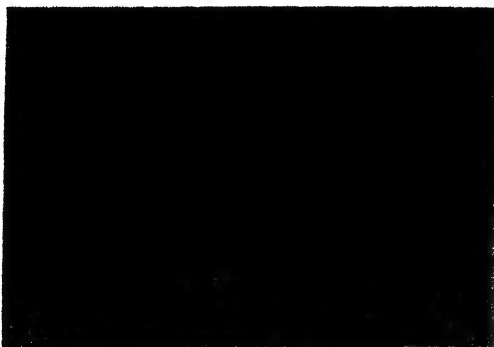


FIG. 178. Uterus A was taken from a virgin rat about fifteen months old which had received a diet deficient in vitamin E for about a year. Note the discoloration which contrasts with the normal colour of uterus B, taken from a control of the same age and history, which had received a supplement of two drops weekly of a preparation of the unsaponifiable matter of wheat germ oil.

longed deprivation of the vitamin the oestrous cycle became abnormal, and it was impossible to render the animals pregnant." This failure to induce pregnancy may have been due to changes in the uterine wall, since there was a degeneration of the smooth muscle and brown discoloration due to small yellow granules in the muscle cells. The uteri of rats which had been pregnant were often large and misshapen and metritis, salpingitis, and ovarian cysts were common. Hessler [158] also noted similar changes in the uterus which occasionally spread to the vagina and ureters. The reaction of the uterine muscle was normal both to drugs acting on it directly and to those acting through its nerve supply. Barrie [50] has recorded pigmentation apparently increased by pregnancy, and also fibrosis of the uterine muscle, and, in some cases, fibromyomata. The longer animals were deprived of vitamin E, the larger the amount they required for a normal pregnancy. A resorption pregnancy also increased the requirements of vitamin E. Females deprived of the vitamin tended not to mate. Vitamin E did not reduce the discoloration of the uterus unless pregnancy occurred—presumably because the increased uterine circulation of pregnancy is necessary to remove the pigment. The pigment is further discussed on p. 728.

Reproduction in the Female. Evans and Burr [2] stated that: "In (female) animals reared on E-free diets the processes of oestrus, ovulation, fertilization, migration and implantation take place in normal fashion, but the young are never born, resorption occurring instead." This sterility, unlike that of the male, could always be promptly cured by giving vitamin E. Later work, however, has modified these statements. Martin and Moore [49] report that "after prolonged deprivation of the vitamin the oestrous cycle became abnormal, and it was impossible to render the animals pregnant." This failure to induce pregnancy may have been due to changes in the uterine wall, since there was a degeneration of the smooth muscle and brown discoloration due to small yellow granules in the muscle cells. The uteri of rats which had been pregnant were often large and misshapen and metritis, salpingitis, and ovarian cysts were common. Hessler [158] also noted similar changes in the uterus which occasionally spread to the vagina and ureters. The reaction of the uterine muscle was normal both to drugs acting on it directly and to those acting through its nerve supply. Barrie [50] has recorded pigmentation apparently increased by pregnancy, and also fibrosis of the uterine muscle, and, in some cases, fibromyomata. The longer animals were deprived of vitamin E, the larger the amount they required for a normal pregnancy. A resorption pregnancy also increased the requirements of vitamin E. Females deprived of the vitamin tended not to mate. Vitamin E did not reduce the discoloration of the uterus unless pregnancy occurred—presumably because the increased uterine circulation of pregnancy is necessary to remove the pigment. The pigment is further discussed on p. 728.

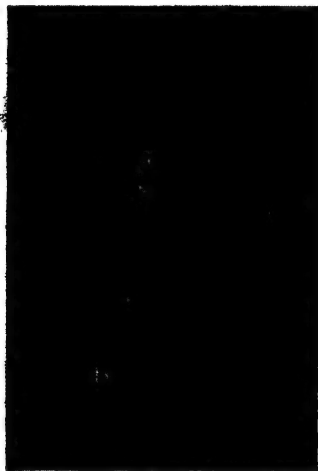


FIG. 179. Uterus A was taken from a normal rat which had been used for breeding purposes while receiving an adequate mixed diet. Uterus B was taken from a rat which received a diet deficient in vitamin E and which had undergone pregnancy during routine vitamin E tests over a prolonged period. It is not only discoloured but permanently enlarged and misshapen.

Bacharach [51] has emphasized the increased requirement of vitamin E after a resorption pregnancy, and has also noted that in such animals the implantation rate is very low. The presumable explanation of the latter is the change brought about in the uterus itself from lack of vitamin E and foetal resorption. The latter is the important factor, since a moderately prolonged deficiency in virgin rats has no such affect.

From all this it can be seen that lack of vitamin E exerts a profound influence on all the sexual mechanism in the female, and not merely on the foetal tissues as was originally held by Evans and Burr [2].

The following account of the pathology of resorption gestation is chiefly taken from the classical work of Evans and Burr [2].

Until the eighth day of gestation no abnormality can be demonstrated by examination of the uterus or its contents, but some irreversible change has already taken place by the fifth day, since vitamin E deficient rats cannot produce living young if a supplement of vitamin E, however large, is given later than the fifth day of pregnancy.

From the eighth day onwards foetal development lags behind the normal by about one day. Mesodermal structures, especially, develop slowly. The hæmopoietic system is greatly affected, the mesodermal blood islands of the yolk sac being few in number. They also disappear early before the liver has adequately taken over the formation of the blood cells. This causes in most embryos, though not in all, a gross anæmia.

In the yolk sac there is a marked reduction in the size and number of the entodermal villi, and the growth and differentiation of the allantois is delayed. The foetal capillaries are generally small in number and only carried a short distance by the allantoic projections. The maternal placenta remains relatively normal apart from being small and having enlarged blood vessels. There is also in the early stages some placental hæmorrhage which appears in vaginal smears.

After the death of the foetus, which occurs about the thirteenth day, but may be later if the diet contains some vitamin E, complete resorption of the foetus slowly occurs. The placenta, however, continues to grow for a few days. Barrie [52], by giving small amounts of vitamin E, has prolonged gestation and delayed resorption until the twenty-eighth day.

The death of the foetus appears to be due to starvation and asphyxia brought about partly by the failure of the blood forming organs and yolk sac, partly by the failure of the allantois.

In eggs from vitamin E deficient hens the embryo develops slowly and dies toward the end of the first week because its nourishment is cut off by a lethal ring in the blastoderm formed by cellular proliferation in the mesoderm [42, 48].

Toxæmias of Pregnancy. Most of the work on the effect of vitamin E on pregnancy toxæmias has been done on man (p. 748). Earrie [53], however, has reported thirty-eight cases in rats in six hundred and sixty-seven pregnancies. All these cases occurred in vitamin E deficient rats, and nearly three-quarters in rats which had been only partially deprived of vitamin E. Barrie states that the toxæmia was "associated with the resorption of foetal material brought about by the inability of the animal to continue the gestation owing to the lack, but not the complete deficiency,

of vitamin E." This is of clinical interest, as in pregnant women a deficiency of vitamin E is unlikely to be ever complete.

Lactation. Whether vitamin E has any effect on lactation is uncertain. From Barrie's work [50] it would appear that some rats who have had only just sufficient vitamin E to carry pregnancy to term cannot suckle their young, though most can. There is some clinical evidence that lack of vitamin E reduces the amount of human milk (p. 747). The amount of vitamin E in the milk of course depends on the amount in the maternal diet (p. 716).

Reproduction in the Male. The following account is taken from the work of Mason [85] and Evans and Burr [2]. In male rats born of mothers on normal diets, and then at weaning placed on deficient diets, sterility occurs when the rats are fifty to one hundred and fifty days old. If, however, such rats are also suckled by mothers on a deficient diet they never become fertile (see also p. 716).

In the first stage of the sterility the sperm appear completely normal both as regards their morphology and motility. Nor can any changes be seen in the testes. Even at this stage, according to Mason [85], the sterility cannot be cured nor complete degeneration of the testes averted. Evans and Burr [2], however, cured about one-quarter of their rats when testicular changes were already marked (Fig. 180).

As the degeneration of the testes progresses the sperm lose their motility and then begin to fuse together, or appear as fused cytoplasmic masses with fused sperm tails. After this no more sperm are produced, and the germinal epithelium of the testes continues to degenerate. This degeneration is first seen in the most mature cells, leading to fusion of the mature sperm. Then the spermatids and secondary spermatocytes show nuclear changes with liquefaction and segregation of the chromatin material. This is followed by the cells fusing together to form giant cells containing as many as forty nuclei. These giant cells tend to slough into the lumen of the tubule. Similar changes occur in the spermatogonia, but the Sertoli tissue undergoes little change. There is a marked difference in the rapidity of the degeneration in different tubules. The testicular changes brought about by deprivation of vitamin E are typical and never seen in any other condition [85]. Sexual interest is preserved for a long period after the onset of sterility, but is at length lost [2, 85, 54] as it is in the female (p. 720).

Similar testicular changes have been reported in the dog [27], guinea-pig [222] and fowl [42], and Mason [85] believes he has also seen them in human testes.

The Relation of Vitamin E to the Endocrine Glands. Drummond, Noble and Wright [54] reviewed the work on the relationship between vitamin E and the endocrine glands in 1989 and reported the results of extensive investigations of their own. They stated that "the experimental results which have been obtained do not indicate that the endocrine system is primarily responsible for the changes observed after feeding rats on an E deficient diet."

The testicular degeneration described previously was again confirmed by the above authors, who found that its progress could not be arrested

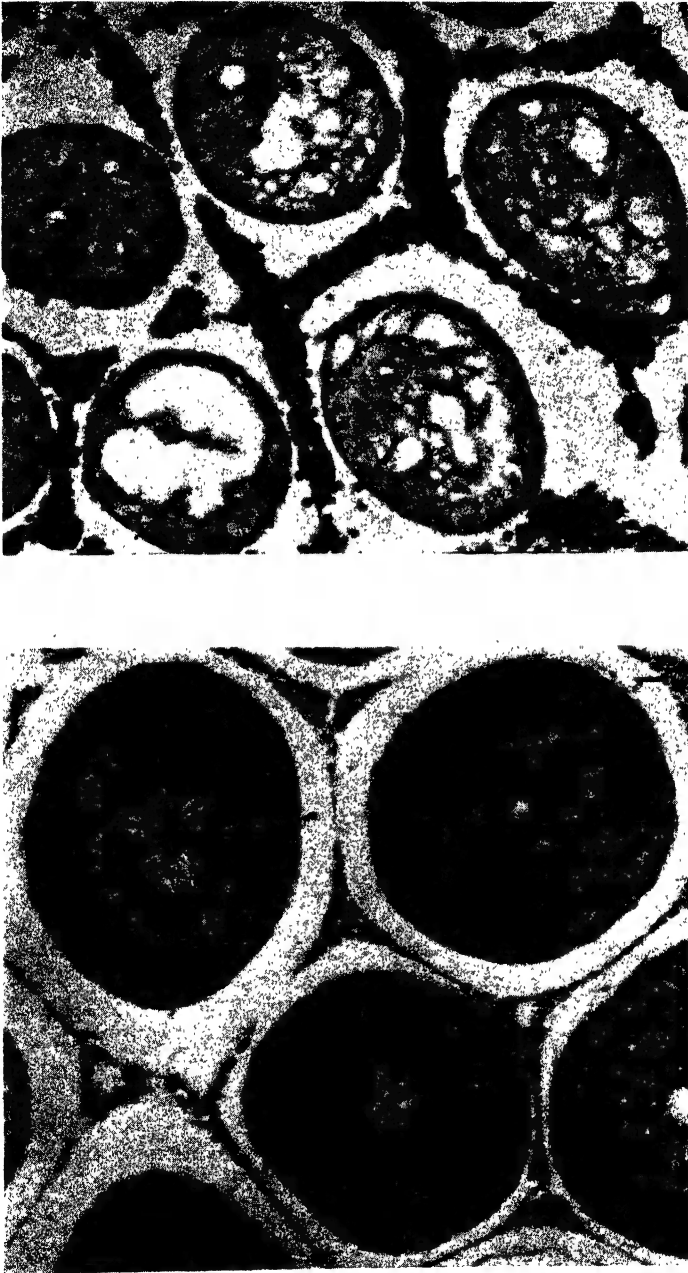


FIG. 180. Microphotographs depicting the testes of litter mate brothers 277 days old fed on an identical vitamin E low diet, save that one brother (photograph 1) also received 0.75 mg. of alpha-tocopherol six times weekly. (For details, see text, p. 722.)

with vitamin E concentrates alone or in combination with injections of extracts from human pregnancy urine or pregnant mare serum. The latter when injected into vitamin E deficient hypophysectomized rats

also had no effect on the germinal epithelium, though the interstitial tissue responded. That the interstitial tissue of vitamin deficient animals continued to function is shown by the weight of the prostates and seminal vesicles remaining relatively normal. Sexual interest, however, was ultimately diminished which has been noted by others [2, 85]. Mason [85] agrees that the testicular damage is not a secondary effect from the pituitary, since the latter's removal does not cause the typical degeneration seen after deprivation of the vitamin. Adamstone [158] reports that vitamin E reinforces the action of testosterone in caponized male fowls.

Thyroid hypoplasia has been reported by Barrie [52] and Singer [55] in rats, and by Anderson and others [44] in dogs. Singer [55] found the hypoplasia did not respond at all to iodine, and so little to injections of pituitary extract that she did not think it a pure anterior pituitary effect. Mason and Bryan [56], on the other hand, could not find any changes in the thyroids of young deficient rats, nor could Telford and his collaborators [57] in rats at any age. There appears to be no explanation of these contradictory reports.

The pituitary both in function and structure has been reported to be affected by lack of vitamin E. Barrie [52] and Underhill [7] state that the anterior pituitaries of both young and old rats show degranulation of the acidophils, a spongy appearance of some of the basophils, and considerable numbers of empty cells which are presumably acidophils. They think that a deficiency of vitamin E hinders the formation of the gonadotropic, thyrotropic, and galactotropic hormones. This would explain the condition of their young rats which had very soft, poorly ossified skulls and cretinism with hypoplastic thyroids; it also explains the difficulty adult rats may have in suckling. Singer [55], however, failed to prevent the thyroid hypoplasia with pituitary extracts and mammary regression occurs only *after* foetal resorption [226]. Most workers also stress the normal appearance of young rats even when these have muscular dystrophy.

Nelson [58] and Mason [85] have reported "castration changes" in the pituitaries of vitamin E deficient male rats, leading to an increase in the factor for stimulating the female genital system in young rats. Since no such changes were found in the pituitaries of female rats, those of the males were thought to be secondary to the testicular degeneration. Rowlands and Singer [59], however, tested the activity of pituitary extracts from female deficient rats on rabbits in oestrus, instead of young rats. From this they deduced that lack of vitamin E caused a decrease in the luteinizing or ovulation producing substance but not in the follicle stimulating substance. These results have been largely confirmed by Drummond, Noble, and Wright [54] using hypophysectomized rats as the test animals. Testosterone is reported to delay the onset of muscular dystrophy in vitamin E deficient rabbits [224]. Minot and Dodd [159] have reported that the urines of seven boys with muscular dystrophy all contained oestrogens while the urines of normal boys did not.

Bacharach and Chance [60] found alpha-tocopherol, when given by mouth or by injection, had no oestrogenic effect in rats. They also state that Underhill has not been able to repeat his observation that alpha-

tocopherol causes oestrus in infantile mice [7]. Beerstecher [61] reports that the oestrogens in the urine of vitamin E deficient rats is normal during their pregnancy until resorption occurs, when it drops sharply. Drummond and others [54] observed no effect, from giving vitamin E, on the genital organs of young rats or those of adult hypophysectomized rats, and Zondek and others [225] failed to stimulate spermatogenesis in immature rats. The work of Shute on the balance between oestrogens and vitamin E in the blood is discussed on p. 745.

Kepinov [62] has observed that in frogs vitamin E is necessary for the formation of the glycogenic hormone of the anterior pituitary. In starved or hypophysectomized frogs injections of adrenalin cause no mobilization of glycogen from the liver, but after vitamin E is given adrenaline produces glycogenesis in the starved but not in the hypophysectomized frogs.

The resorption gestation which always occurs in vitamin E deficient rats cannot be prolonged to term by giving injections of whole pituitary glands, by implanting two fresh pituitary glands every other day or tablets of progesterone or testosterone, or by injections of extracts of pregnant mare serum or human pregnancy urine [54]. Ershoff [226] likewise failed to prevent resorption with oestrone, progesterone or the lactogenic hormone. The corpora lutea only degenerated after the death of the foetuses, and so cannot play any part in the resorption. Mammary regression also only occurred after foetal death. Clinical work on the relation of vitamin E to the corpus luteum is discussed on p. 746.

The weights of the pituitary, adrenals, ovaries, and uterus of deficient animals remain normal, while those of the prostate and seminal vesicles are not greatly reduced compared to the diminution of the testes [54]. A longer list of organs is given by Copping and Korenchevsky [7], who also report that the thymus is larger in both male and female vitamin E deficient rats. Tonutti [256] has reported involution of the adrenal cortex.

"From a consideration of the results obtained it appeared very unlikely that the effects of E-deprivation could be explained by an alteration in function of the anterior pituitary gland, the slight changes recorded being in all probability related to a secondary effect on the pituitary" [54].

The Relation of Vitamin E to Growth. Impaired growth is one of the earliest symptoms of a deficiency of vitamin E both in the embryo (p. 721) and the young animal. Evans and Burr [2] noted that the growth of rats was affected by the amount of vitamin E in the diet, and Copping and Korenchevsky [7] state that "the final body weight, total gain in weight and amount of fat deposition were less in the rats deprived of vitamin E than in the corresponding controls." Barrie [52] has emphasized the poor growth of her young rats, and Nelson and others [68] have reported that on diets deficient in vitamin E rats stop growing, to begin again when alpha-tocopherol is given. Emerson and Evans [64] bred four generations of rats on diets low in vitamin E. Each successive generation grew less than the last.

Female mice, according to Menschik [258], cannot store fat while on a diet rich in fat but deficient in vitamin E, though animals receiving

2.5 mg. daily of vitamin E store excessive fat in all the normal depôts and also in the liver.

Rabbits may cease to grow days before any other symptoms of a deficiency appear (p. 781). Foxes [45], dogs [44] and children (p. 765) also probably require vitamin E for proper growth.

The Relation of Vitamin E to Muscular Dystrophy. A degeneration or dystrophy of the voluntary muscles occurs as the result of insufficient vitamin E in the rabbit [18, 82], guinea-pig [72], rat (p. 781), hamster [210], mouse [188], dog [27, 44], the sheep and goat [212], the tree kangaroo [29] and duck [39] and possibly man (p. 747). A degeneration of smooth muscle is seen in the gizzard of the turkey [40] and in the uterus, vagina, ureters and seminal vesicles of the rat [49, 158]. The effect of lack of vitamin E on cardiac muscle is discussed below.

How far the muscular dystrophy is a *primary* effect of lack of vitamin E and how far it is a *secondary* effect brought about by changes in the nervous system is obscure. The subject is discussed on p. 781.

Muscular dystrophy in animals will be discussed under the headings of (a) the clinical picture, (b) the muscular degeneration, (c) the changes in metabolism, (d) rancid fats: their destruction of vitamin E and their relation to muscular dystrophy, (e) the cure of muscular dystrophy.

The Clinical Picture. Evans and Burr [8] first reported a mysterious paralysis which appeared in the suckling young of rats deprived of vitamin E. Suddenly, towards the end of weaning, the young, who till then had appeared normal, became weak, lost weight, and sometimes died in a few hours. A few animals escaped altogether, and some spontaneously recovered without treatment. Others again did not die but remained in good health though partially paralysed. In adult rats muscular dystrophy (Fig. 183) develops very slowly [5, 6, 49]. But the rat is not a satisfactory animal to use when investigating muscular dystrophy because a nervous degeneration may obscure the picture (p. 781).

Guinea-pigs and rabbits, on the other hand, develop muscular dystrophy without any neurological complications (p. 781). The condition appears within a few weeks on a deficient diet and, unless vitamin E is given, always progresses rapidly to death. Generally the onset of muscular dystrophy prevents deficient rabbits from reaching sexual maturity, but in a few dystrophic animals Pappenheimer [29] has reported the birth of living young, and here muscular dystrophy was present at birth. It is interesting to note in passing that Mackenzie and McCollum [18] point out that experiments purporting to show rabbits do not need vitamin E for reproduction must be fallacious as the mothers would die of dystrophy before complete depletion of vitamin E could prove it unnecessary for reproduction.

Cardiac failure, in spite of the absence of pathological changes in the cardiac muscle [227], appears to be the reason for the sudden death of animals with muscular dystrophy: a death which it is hard to explain by the condition of the voluntary muscles. Houchin and Smith [228] have shown, by well controlled work, that dystrophic rabbits die from doses of posterior pituitary extracts which are harmless to normal rabbits. On the other hand dystrophic rabbits not only tolerate doses of digoxin and

ouabain which are normally lethal, but also have their lives prolonged by such doses. These findings appear to be only explicable on the assumption

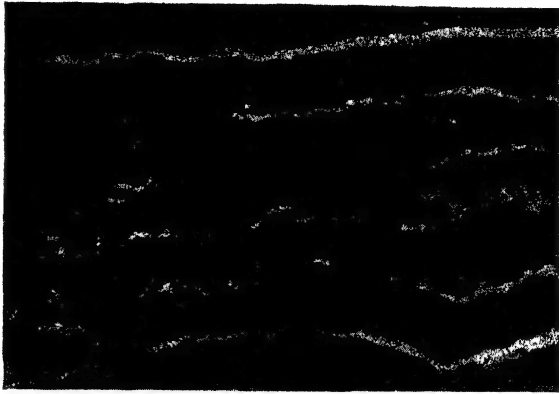


FIG. 181. Microphotographs of muscle removed for biopsy from the calf of an English boy aged seventeen with pseudohypertrophic muscular dystrophy. In the top fibre transverse striations are barely evident and the left-hand portion shows well wrinkling and longitudinal fraying. In the second fibre these changes are more marked: transverse striations have completely vanished, wrinkling and fraying are very extensive and there is considerable sarcolemma nuclei proliferation. The other fibres show varying degrees of similar change.



FIG. 182. Further microphotograph of the muscle in Fig. 181. The middle fibre shows on the left a portion of healthy muscle fibre with well-marked striations, a very rare finding. The right-hand portion of the fibre shows swelling and hyaline change with complete loss of the striations.

that the heart of the dystrophic rabbit is so severely damaged that the decrease in the cardiac circulation brought about by the pituitary extract precipitates cardiac failure while the different toxicity of the cardiac

glucosides in the normal and dystrophic animal is that which would be expected if they were acting upon a normal or upon a failing heart. Houchin and Smith suggest that lack of vitamin E may be a factor in the sudden cardiac failure of beriberi, and may also be of importance in the nutrition of the human heart. It is interesting to recall that Moore and Wang [229] have reported that the pigment in senile brown atrophy of the heart in man is similar to that found in the dystrophic muscles of animals, which is described in the next section.

The Muscular Degeneration. Goettsch and Pappenheimer [4] in 1931 first described what they termed "nutritional muscular dystrophy" in guinea-pigs and rabbits when these animals were deprived of vitamin E. They wrote " . . . in the more chronic cases . . . these muscles with replacement fibrosis and lipomatosis closely resemble those of progressive muscular dystrophy in man." In 1940 Pappenheimer [29] in an excellent review stated: "The disease may run a chronic course. In such animals comparatively few fibres are destroyed at any one time, but their gradual loss and replacement by fat and fibrous tissue brings about a picture which is identical with that of an advanced case of human muscular dystrophy." The pathological changes in the muscles of young rats have been described by Olcott [227] (see Figs. 181 and 182).

Not all muscle fibres degenerate at the same time, some remaining normal while others rapidly disintegrate. The first change in the affected fibres is loss of their transverse striations, and a hyaline change, sometimes going on to complete degeneration. Polymorphs and histiocytes appear, the latter often fusing into giant cells. Many fibres make attempts at regeneration; in some the nuclei increase and the muscle substance splits longitudinally, making fresh small fibres; in others the nuclei multiply rapidly under the sarcolemma, so that a tube of nuclei is formed. On the surface new myofibrils start to reform new muscle fibres. These efforts at regeneration go on even in the midst of the most intense degeneration [4, 29, 49]. Hoagland and others [254] have described the changes in human dystrophic muscles shown by ultra-violet photomicrography.

Martin and Moore [49] observed small yellow granules in the degenerating muscle fibres of rats with chronic muscular dystrophy which coloured the whole muscle brown. This discoloration was also observed in the uterine muscle (p. 720) and has been shown by Moore and Wang [229] to give a characteristic yellow or bronze-coloured fluorescence when exposed to the irradiation of a mercury arc lamp, screened with Wood's glass. Even before the muscle appears noticeably discoloured such fluorescence is well marked. The fluorescent substance has been concentrated, and a similar substance has been isolated from the brown atrophied heart of an old woman. The possible clinical implications of this have been discussed in the preceding section.

Changes in Metabolism. The metabolic changes brought about by lack of vitamin E are discussed on p. 717, so that here it is only necessary to draw attention to the way in which the creatin in the urine reflects the disturbed metabolism of the muscle. Just as dystrophic changes may be found in the muscles while the animal yet appears normal [82, 85], so may the creatine rise in the urine before any clinical change can be seen. Ni [67]

was the first to apply to research in muscular dystrophy the well-known fact that urinary creatine is increased when the volume of functioning muscle is decreased [86]. He and Mackenzie and McCollum [18] noted the warning rise in the creatine of animals deprived of vitamin E. The latter authors have very fully investigated the subject and report that the best guide to the approach of muscular dystrophy is the rise in urinary creatine. This may occur while the increase in weight and the appetite are still satisfactory.

The drop in creatine brought about by giving vitamin E is dramatic and precedes clinical improvement in strength, appetite and weight. It occurs in from twenty-four to forty-eight hours [18, 82].

Rancid Fats: their Destruction of Vitamin E and their Relation to Muscular Dystrophy. Rancid fats rapidly destroy vitamin E by oxidation. This destruction is most liable to occur when vitamin E is in the form of the synthetic vitamin or concentrated preparations, since then it is no longer protected against oxidation by the anti-oxidants found associated with it in such natural sources as whole wheat germ [76].

It is important to realize that as vitamin E itself is an anti-oxidant it will be destroyed by fats before they become rancid through auto-oxidation. In other words a fat need not be rancid, certainly not smell rancid, before all its vitamin E is destroyed. Further, rancid fats can destroy vitamin E during digestion as long as both are fed together or within a short time of one another [87, 82, 88]. Claims that rancid fat given by mouth or by injection destroys vitamin E already absorbed into the body [77, 78] require further confirmation, probably being incorrect [79, 202]. If substantiated they are an argument against using ketogenic diets in treatment.

The destruction of vitamin E by fats, due to their auto-oxidation, has been investigated by Cummings and Mattill [80], who state that "the oxidation of unsaturated fats by atmospheric oxygen causes the formation of substances which impart to those fats a characteristic acrid odour, usually described as rancid." Rancidity, however, is difficult to define: some of its products are free fatty acids, aldehydes, ketones, and peroxides. Some substances having hydroxyl groups, such as wheat germ and other vegetable oils, retard rancidity and so protect vitamin E, but ultimately wheat germ oil itself becomes rancid. The fats commonly used in food are auto-oxidizable in the following order: cod liver oil, lard, butter. Margarine is compounded of various fats, and sometimes has a basis of soured skim milk (see also pp. 654, 789).

From the point of view of the diets used to produce muscular dystrophy this destructive action of fats is most important. Not realizing this has led to much confusion. When Goettsch and Pappenheimer [4] first reported nutritional muscular dystrophy, they did not consider a deficiency of vitamin E was the cause because wheat germ oil supplements failed to prevent the dystrophy. It now can be seen that this was because their diet contained so much cod liver oil and lard that the vitamin taken in the wheat germ oil was destroyed during digestion in the stomach [82].

Ni [68] also made a perplexing observation, when by adding Chinese donkey skin gelatine (ah-chiao) to a vitamin E deficient diet he prevented

and cured muscular dystrophy. This not only lent support to, but also gained support from, the treatment of human muscular dystrophy with gelatine (p. 764). The explanation, however, according to the further work of Ni [70], is that the *ah-chiao*, being added to the diet as a powder, adsorbed pro-oxidants and so prevented the destructive oxidation of vitamin E.

The most serious result of not realizing the effects of rancid fat was that Morgulis and Spencer [81] were led to postulate that two factors were necessary to prevent or cure muscular dystrophy. One was fat soluble and probably vitamin E, the other was water soluble being found in wheat germ and lettuce. The evidence for this came from observations that wheat germ oil alone could not prevent dystrophy but wheat germ oil and lettuce or whole wheat germ could do so. Much work has been devoted to investigating this "dual theory" of muscular dystrophy, which has now been proved wrong.

The most thorough proof that deprivation of vitamin E is the sole cause of muscular dystrophy is given by Mackenzie and others [82] using the same diet as Goettsch and Pappenheimer. Alpha-tocopherol given separately from the rancid food, so that no destruction of the vitamin could occur, prevented muscular dystrophy. Wheat germ from which vitamin E had been extracted (defatted wheat germ) had no effect. Further, alpha-tocopherol cured animals who had had no "water soluble factor," which proved that the cure was not due to stores of the latter in the body. To complete the destruction of the "dual theory" of muscular dystrophy it was shown that animals who received wheat germ oil mixed with the rancid ration became dystrophic, but if defatted wheat germ was added as well dystrophy did not occur: the defatted wheat germ protected the vitamin E from oxidation. This rounds off the proof that vitamin E is the only vitamin necessary to prevent muscular dystrophy and that the superiority of wheat germ over vitamin E alone, or as wheat germ oil, is solely due to its protecting vitamin E from destruction by rancid fats. This, of course, does not mean that other vitamins contained in wheat germ, such as the vitamin B complex, may not be necessary for the proper utilization of vitamin E in the body, in the same way as vitamin E is necessary for the utilization of vitamin A (p. 789). The animals discussed above were all on diets containing an abundance of all the vitamins except vitamin E.

The Cure of Muscular Dystrophy. Vitamin E cures and prevents muscular dystrophy in rabbits and guinea-pigs and various other animals, but it can only prevent and not cure dystrophy in adult rats (p. 781). In the previous section it has been seen that the vitamin must be given so that it does not come in contact with rancid fat either in the food or in the stomach. Where this may, however, occur the vitamin should be given as whole wheat germ, because in this form it is given some protection against oxidation. Apart from this it is unimportant what preparation of vitamin E is used—whole wheat germ, wheat germ oil as long as it itself is not rancid, or alpha-tocopherol. The latter may also be given by injection, but is less potent than when given by mouth (p. 714).

Vitamin E acts with amazing rapidity. Within twenty-four hours

the creatine in the urine may drop, and in a couple of days may be normal. The appetite and weight of animals improve as the creatine falls, and a normal rate of growth is re-established in one or two weeks. Even when the animals are so weak they cannot stand or feed themselves vitamin E leads to a rapid return of strength in one or two days, and by the end of a week few symptoms of the dystrophy may be left. The cure of such animals is permanent and complete. All trace of even severe dystrophic changes may vanish from the muscles in a week [18, 29, 82]. In children, however, recovery, if it occurs, may take years (p. 768).

The Relation of Vitamin E to Nervous Degenerations. In rabbits and guinea-pigs, as has been seen in the previous sections, lack of vitamin E causes the rapid onset of a muscular degeneration which can be rapidly cured by the vitamin. It is highly improbable that this degeneration is secondary to any nervous lesions because no changes have been found in the central nervous system [29] and the terminal neurites or end plates in the muscles remain normal even when the muscle fibres have completely degenerated [66]. Further the rapidity of the recovery when vitamin E is given is far greater than could be accounted for by regeneration of nervous tissue. In rabbits and guinea-pigs, therefore, it seems most probable that lack of vitamin E causes a primary muscular dystrophy uncomplicated by any neurological degeneration, though it must be admitted that some workers (p. 788) have reported functional or anatomical changes in the nerves of these animals.

In rats, on the other hand, lack of vitamin E probably affects both the muscular and the nervous systems so that a primary muscular dystrophy and a secondary muscular degeneration occur together. The paralysis of rats, however, is a complicated problem, partly because some observers report extensive degeneration of the nervous system while others do not, partly because young deficient rats may spontaneously recover without vitamin E and partly because adult rats, though they develop paralysis very slowly, cannot be cured by vitamin E nor even have the progress of their paralysis checked. This inevitable progress of the paralysis is more typical of a neurological than a muscular disorder and is entirely unlike the primary muscular dystrophy of the rabbit and guinea-pig, which can be rapidly cured.

The outstanding work on the nervous system in deficient adult rats is that by Ringsted [5] in 1985 and Einarson and Ringsted [6] in 1988. At the date of their experiments pure synthetic alpha-tocopherol was not available so wheat germ oil had to be given to their control animals. Apart from this probably unimportant point, their work and their histological technique appears to be beyond criticism. They reported that the initial lesion is a degeneration, in the lumbar cord, of the proximal parts of the posterior roots and the proprioceptive paths in the posterior tracts and probably in the uncrossed tactile paths. The amount of neuroglial reaction is inconstant, depending apparently on the rapidity of the degeneration. The cells of the spinal ganglia are not affected. Degeneration of the anterior horn cells of the lumbar cord generally begins shortly after the degeneration of the posterior columns and progresses until it is more or less complete. With the death of the motor cells the motor fibres of the peripheral nerves

degenerate and the muscles atrophy. The pyramidal tracts might be expected also to be constantly affected instead of only slightly and rarely, but these tracts are of very recent origin in the evolution of the nervous system, being unimportant in the rat and not really analogous to those in man. As it is, "We have, on the whole, a pathologic-anatomical picture that resembles closely a combination of two of the most important systemic degenerations occurring in man, namely: *tabes dorsalis* and spinal progressive muscular atrophy" [6]. If the rat had true pyramidal tracts, the picture would be complete. Einarson and Ringsted [6] failed to prevent

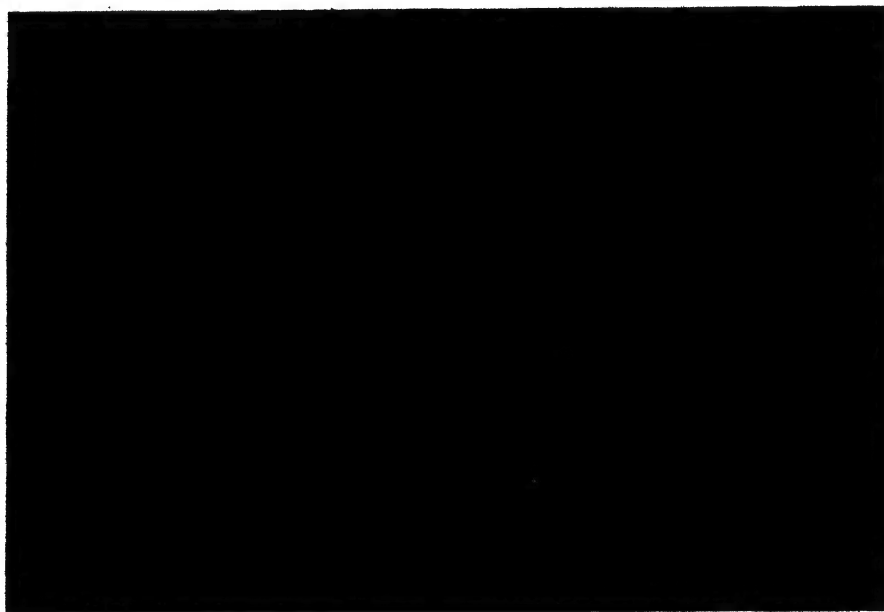


FIG. 188. The rat in the right partition is a virgin female which received a diet deficient in vitamin E for about thirteen months. It is emaciated, rough haired and has paralysed hind legs. The rat in the left partition is a control animal which received 3 drops weekly of the unsaponifiable matter of wheat germ oil.

the progress of the nervous degeneration by giving vitamin E, except possibly in its earliest stages.

Other workers such as Monnier [6] and Gutierrez-Mahoney [212] have confirmed that lack of vitamin E causes a widespread degeneration of the central nervous system of adult rats, though their descriptions of the lesions differ and also do not exactly tally with those given by Einarson and Ringsted. These discrepancies between the reports of various workers do not invalidate their essential agreement, since differences in diet, and so in the rapidity of the neurological degeneration, will considerably affect the final condition of the nervous system. This was originally investigated and emphasized by Einarson and Ringsted [6]. Lipschütz [75] has described similar changes in the nervous system of the young paralysed rat.

On the other hand, Olcott [227], Pappenheimer [29] and Wolf and Pappenheimer [280] have all failed to find any changes in the nervous system of young or old paralysed rats. There seems no adequate explanation of these negative findings.

The motor end plates in the muscles of paralysed rats have been found to be normal by Pappenheimer [29] and few in number by Telford [75]. Confirmation of the latter's work comes from the observations of Hines and others [204], who showed that both in dystrophic rats and guinea-pigs the tension in muscle developed after direct electrical stimulation was greater than that after stimulation of the motor nerve. De Castro and others [284] claim that in rabbits changes in the central and peripheral synapses precede the muscular degeneration.

Cutting the motor nerve of a muscle protects it from the onset of dystrophy [29, 204]. This effect cannot be due to any removal of an abnormal nervous stimulation since it also occurs when the tendon is cut [29]. This strongly suggests that vitamin E is necessary for the active but not the resting muscle.

Neuromuscular regeneration after crushing of a motor nerve is not delayed by a deficiency nor hastened by a super-abundance of vitamin E [204].

Wheat germ oil is reported to give protection against the neurological symptoms of distemper and diphtheria (p. 737).

Davison's extremely important investigations (p. 774) on human amyotrophic lateral sclerosis also strongly suggest that vitamin E is intimately connected with the central nervous system. He treated ten cases with wheat germ oil and alpha-tocopherol, and in six of these post-mortem histological studies (Figs. 192 to 205) showed that, in comparison with untreated cases, there was less destruction of axis cylinders and myelin sheaths and considerably less gliosis. There was no difference in the nerve cells of the bulbar nuclei and anterior horn cells.

Vitamin E Deficiency in Chicks. In chicks lack of vitamin E causes two quite separate conditions—nutritional encephalomalacia and the "alimentary exudative diathesis."

Nutritional Encephalomalacia. This was first described by Pappenheimer and Goettsch [87] in chicks fed on diets deficient in vitamin E. The chicks thrive for about three weeks and then suddenly or slowly developed weakness, ataxia, tremors, retraction of the head and other symptoms pointing to some nervous disorder. Death was common, but about two-fifths of the chicks never developed the disease and others recovered without treatment, in the same way that young rats with no treatment may recover from paralysis (p. 726). As the birds grow older the condition becomes rarer, never being seen in the adult [29].

All parts of the brain may be affected, but the severest lesions are generally found in the cerebellum. There is extensive ischaemic necrosis with oedema, small haemorrhages, and hyaline thrombi in the small vessels in and about the necrotic areas. The brain elements are disrupted, with degeneration and necrosis of the cells. Pappenheimer [29] states that while the arrest of the circulation is the cause of the condition it is impossible to tell whether the closure of the vessels was originally functional

or caused by the hyaline thrombi. The cholesterol content of the brain is said to be decreased [153], but this is probably incorrect [214].

Dam and his collaborators [41] have shown that synthetic alphatocopherol protects chicks against this condition. Ni [69] reported that ah-chiao also gave protection, but he later found this was due to the ah-chiao preventing destruction of vitamin E by the rancidity of the diet (p. 729). Failure to produce the condition by diets in which vitamin E has been oxidized by ferric chloride [29] is presumably due to the failure of the ferric chloride to destroy all the vitamin [88].

Alimentary Exudative Diathesis. This is the name given by Dam and Glavind [19] to a condition which occurs in chicks reared on a diet low in vitamin E. The description given by these authors and by Bird and Culton [90] is of a generalized œdema with fluid of the composition of blood plasma collecting in the subcutaneous and subfascial tissues, especially those of the breast and abdomen. The fluid also collects in the pericardial and peritoneal cavities. The brain and lungs are œdematous, and there is coronary and intestinal hyperæmia. Deposits of urates are found in the kidneys and ureters. This œdematous condition is reminiscent of that found by Pappenheimer [29, 88] in young mice and rabbits born with muscular dystrophy.

The condition appears between the sixth and thirtieth day. Not all chicks are affected; those that are die in six to eight weeks. Protection is given by synthetic vitamin E, dehydrated grass, wheat germ oil or alcoholic extract of dried hips. The latter appears to contain more vitamin E than does wheat germ oil, judging by the protection it affords. Dam and Glavind [19] have suggested assaying the vitamin E content of foods by their protective action against this œdematous condition.

Dam [214] in 1944 reviewed his own work and that of others on these two deficiency diseases of chicks, especially from the point of view of what factors decide which disease shall develop. The amount and kind of fat in the diet is extremely important. On fat free diets exudates seldom develop and encephalomalacia never. Both conditions are accentuated by highly unsaturated fats; fresh cod liver oil, linseed oil and lard especially causing exudates while the fatty acids from hog liver cause encephalomalacia. Oleic acid and thoroughly rancid cod liver oil have no effect, but a mixture of rancid and fresh cod liver oil has the same effect as fresh cod liver oil. This shows that it is the unsaturation of the fats and not their rancidity which is important. An increase in the sodium chloride of the diet increases exudates and so does a certain carbohydrate protein ratio and also cholesterol, though the latter gives protection against encephalomalacia. Inositol gives protection against both conditions.

Dam believes, from the similarity of lipocæic to vitamin E in its effect on the plasma lipoids and from the partial protection which it gives against exudates, that the formation of lipocæic depends on vitamin E.

Ni [70] reports that large amounts of vitamin A and carotene, and possibly vitamin D, increase the incidence of encephalomalacia.

The Relation of Vitamin E to other Tissues: Neoplasms: Resistance to Infection. *Fur and Skin.* The fur of rats on a diet low in vitamin E is reported to remain infantile and unusually white in the young [52], while

in the adult it becomes coarse, sparse, and discoloured [64] (Fig. 188). Skin sores have often been reported in rats [49] and in dogs [27]. We have often found wheat germ most valuable for the coats and health of dogs.

Osseous and Ocular Tissues. Very soft skulls with little ossification have been observed in the suckling young of rats deprived of vitamin E by Barrie [52], and a similar condition in adult rats has been reported in a personal communication by Wright. Very high intakes of vitamin E cause decalcification (p. 718). The eyes of adult vitamin E deficient rats have been said to be unduly protuberant by Demole and Pfaltz [89]. These alterations in the eyes and osseous system are of interest because in human muscular dystrophy analogous changes may occur (p. 747).

Demole and Knapp [89] report that in adult rats a deficiency of vitamin E causes clouding and vascularization of the cornea, keratoconus, iridocyclitis, opacities of the lens and serous retinal exudates. Though such changes have never been reported in any of the vast numbers of rats which have been observed by other workers, yet they are of interest in view of Stone's claim (p. 765) to cure syphilitic interstitial keratitis with wheat germ oil and the vitamin B complex. It may be that Demole and Knapp's rats and Stone's patients were suffering from a mixed deficiency, which in the latter was unmasked by the syphilitic infection.

Dental Degeneration. Irving [283] has described changes in the incisor teeth of rats which he believes only occur from lack of vitamin E. In the centre of the middle third of the tooth there is a sudden premature and abnormal degeneration of all layers of the enamel organ, which is replaced by fibrous tissue. Davies and Moore [97] noticed that the external surfaces of the incisor teeth of vitamin E deficient rats was white, presumably because the deficiency interfered with the metabolism of carotene (p. 739). It is puzzling that the teeth of Irving's rats maintained their normal brown colour.

Renal Degeneration. Martin and Moore [49] have observed a slow progressive parenchymatous degeneration of the kidney unaccompanied by any inflammatory reaction or by any change in the blood vessels. The first renal changes occur after deprivation of vitamin E for three or four months; after ten months nearly all the convoluted tubules are destroyed, so that it is surprising that the animal yet survives. The degeneration starts in the cells lining the convoluted tubules, which become granular and detached from their basement membrane. In time the degeneration may spread to the loops of Henle and the collecting tubules. The glomeruli show little change (Figs. 184, 185).

Neoplasms. No confirmation has been forthcoming for the suggestion of Rowntree and others [91] that some preparations of vitamin E cause malignant tumours. These authors reported that all of fourteen rats given a crude ether extract of wheat germ developed sarcoma of the abdomen. No other wheat germ preparations had this effect. Bryan and Mason [92], Ginzton and Connor [98] and Rider [94] among many others, could not confirm Rowntree's work, the only effect being that the experimental animals of the latter appeared in better health than the stock animals. It seems clear that there is no danger in using wheat germ oil. Davidson [95] concluded that a diet very rich in vitamin E, and also all the other

vitamins, tended to increase the resistance of mice to tar carcinoma, but Hadow and Russell [96] could not demonstrate that wheat

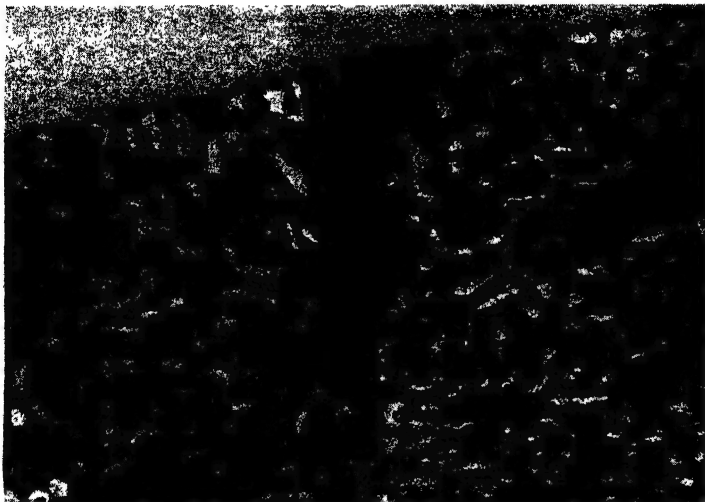


FIG. 184. Microphotograph of normal kidney from a rat given a vitamin E concentrate.

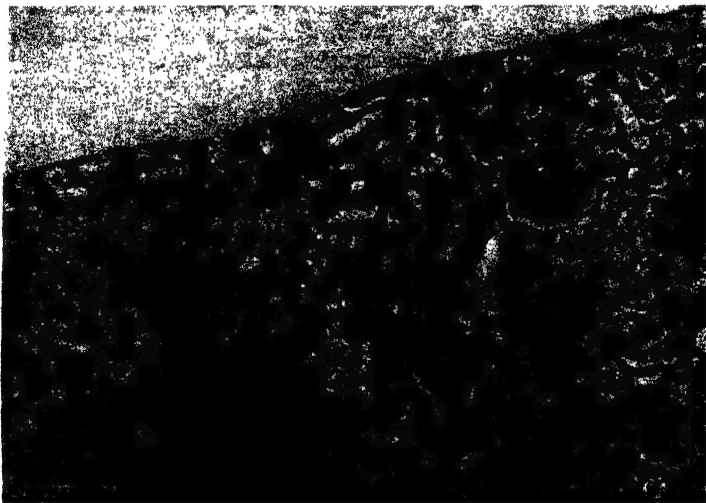


FIG. 185. Microphotograph of kidney from a rat on a diet deficient in vitamin E. Note the extensive degeneration and detachment of the epithelial lining of the urinary tubules.

germ oil had any effect. Adamstone [158] has published work suggesting that cod liver oil causes intestinal sarcoma in fowls deficient in vitamin E.

Resistance to Infection. The only evidence that vitamin E may be

concerned in the resistance of the body to infection comes from Sabin and Duffy [105], who state that lack of vitamin E decreases the resistance of young mice to virus infections and from Vøgt-Møller [281] who gave to two groups of thirty dogs each, on a good mixed diet, either pure tocopherol or wheat germ oil for four days after the first symptoms of distemper. Compared with thirty controls the mortality rate was not affected but the wheat germ oil, though not the tocopherol, decreased the incidence of nervous complications. Butturini [181] states that natural or synthetic vitamin E protects animals against diphtheria toxin and hastens recovery in man. The effect of vitamin E on tabes, syphilitic interstitial keratitis and leprosy is discussed on pp. 775, 765 and 766.

The Relation of Vitamin E to Other Vitamins. The increasing use of synthetic vitamin E in medicine makes the relationship of vitamin E to the other vitamins of considerable practical importance. It is possible that vitamin E needs other vitamins not only for its proper use by the body but also for its destruction when it is present in excessive amounts. If this is so treatment with synthetic vitamin E, without at the same time increasing the consumption of the other vitamins, will be unsatisfactory. Its value will be limited by the amounts of the other vitamins already present in the diet. These are unlikely to be so plentiful that they will be able to do more than aid in the utilization and metabolism of the vitamin E already present.

Little information on the problem is provided by animal experiments, since these mostly aim at giving a large amount of all vitamins other than that being investigated. This prevents any complications arising from a deficiency of the other vitamins, but it has the drawback of hiding whether or no the limiting factor in the use of one vitamin is the amount of another. An example of the complicated interrelation of the vitamins is given on p. 759.

Vitamins A and D. The relationship of vitamin A to vitamin E has already been discussed on p. 42, so that here it is only necessary to reiterate that in human diets the utilization of carotene probably depends very largely on an abundant intake of vitamin E [285, 286]. The effect of vitamins A and D on encephalomalacia in chicks is described on p. 784. The dystrophy of voluntary muscles once thought to be due to lack of vitamin A is now known to be really due to lack of vitamin E [100]. The work of Adamstone [158] suggests a relationship between vitamin D and vitamin E and a different effect of vitamin E on the metabolism of different forms of vitamin D.

The Vitamin B Complex. A great deal of attention was drawn to the vitamin B complex or "a water soluble factor" in its relation to vitamin E by the erroneous belief of Morgulis and Spencer [81] that nutritional muscular dystrophy was due to a deficiency of both vitamins. It now seems clear that the importance of "the water soluble factor" is in protecting vitamin E from oxidation before it is absorbed (p. 780). This, however, does not rule out the possible importance of the vitamin B complex for the utilization and destruction of vitamin E by the body. No experimental work has been done on the effect of deprivation of the vitamin B complex on the utilization of vitamin E. The association of

the latter with the vitamin B complex in nature might suggest some metabolic relationship.

The only possibly relevant investigations are those of Holmes and Pigott [99], who claim, probably erroneously [166], that massive doses of vitamin B₁ permanently cure young rats of muscular dystrophy in one or two days. Vitamin B₆ has not been found to be related to vitamin E, at least as regards the cure of muscular dystrophy in either man (p. 762) or animals [82]. Vitamin E protects biotin and the vitamin B complex against destruction by rancid fats in foods (p. 717). A relationship has been suggested between vitamin E and vitamin K [165].

VITAMIN E IN FOODS

There is little vitamin E in the average English diet. Statements that it is plentiful are founded on the loose generalization that it is found in a great variety of foods. Even if this is true, which is doubtful, it is only present in very small amounts.

Wheat germ and green leafy vegetables, among which lettuce holds a pre-eminent place [2, 25], are the only rich sources of vitamin E, but the germ of the wheat is removed by modern processes of milling and is only grudgingly partly replaced in flour under the compulsion of war legislation. Wheat germ is so valuable for the feeding of live stock that it appears unlikely that it will be left in English bread in peace time, since the nutrition of pigs rather than people is most lucrative. The sorry history of the Government control of the millers [238] holds out little hope for the future. Yet with the removal of the germ the cheapest and only universally eaten source of many of the vitamins is lost. For the poor this is a tragedy: even for the rich the loss of vitamin E is not easily made up by other foods.

"White flour does more than Malthus can
To sterilize the Englishman."

Wholemeal or brown bread or war-time bread made from eighty-five per cent. extraction flour has never become popular, and is often thought to be the only alternative to white bread. Actually another alternative is stone ground bread which is preferred by most people once they are accustomed to it. It is a light cream in colour, contains the germ but no bran, and keeps excellently. Being as bland as white bread, it is suitable for patients with gastritis or "weak digestions." Some "brown breads" contain the germ, but often this has been removed during milling, specially treated, and then returned again to the flour. Such treatment may well destroy some or all of its vitamins. There are no reasons against using stone ground flour in domestic cookery, since it is as good as white flour for pastry and soufflés.

A further great advantage of stone ground flour is that it contains less phytic acid than brown flours, so that there is less danger of calcium, iron, and the other minerals present in the diet forming insoluble phytates in the gut with their consequent loss to the body (p. 640).

Brown rice and ordinary oatmeal both contain the germ of the grain and so would be excellent sources of vitamin E were they eaten regularly.

Green leafy vegetables, the other rich source of vitamin E, are never eaten in large amounts. The poor often cannot afford to eat them at all. Children removed from city slums to the country because of air raids regard cabbage as a new and unpleasant aberration of the uncivilized countryman. Rose hips are very rich in vitamin E [19], but probably the seeds and not the pulp contain the vitamin, so it will be absent in rose hip jam [101].

Guggenheim [286] and Hickman and others [285] have emphasized that *one of the most important rôles of vitamin E in the diet is to ensure that carotene and vitamin A shall be fully utilized*. The difference in the value of carotene from different vegetables depends on the amount of vitamin E which they contain [286]. The theoretical background of this practically important subject has been discussed on p. 42.

The other foods which should contain a small amount of vitamin E are milk and eggs; butcher's meat and fat; and possibly offals and dripping (p. 714). But the vitamin E of both milk and eggs depends on the maternal diet [8, 30, 42, 48], and so may be low when cows are stall fed and chickens are kept cooped on egg farms.

Effect of Rancidity, Staleness, Storage and Cooking. Rancidity rapidly destroys vitamin E (p. 729). This is probably of importance in human diets. Most human foods containing fat are eaten stale, and even if they do not smell rancid may well have lost all their vitamin E, since this will be destroyed before frank rancidity can develop. Foreign meat is often "tainted," and even when it appears fresh its long storage has altered its flavour compared to locally killed English meat. This change in flavour must depend on some chemical change in the meat. Pasteurized milk is often staler than is realized [102], and butter and margarine are seldom fresh. In fact, the foods which should contain a small amount of vitamin E may well contain none by the time they are eaten.

Ordinary domestic cooking will not destroy any large amount of vitamin E unless rancid fat is employed. Little is known of the content of vitamin E in tinned and preserved foods, but bleaching, at least of flour [289], causes considerable loss. There is some evidence that dried and pasteurized milks contain no vitamin E [185], so artificially fed infants may receive inadequate supplies (p. 765).

Preparations of Vitamin E available for Medical Use. Vitamin E may be given as synthetic alpha-tocopherol, as wheat germ oil, or as whole wheat germ. The latter is by far the best.

Synthetic Alpha-Tocopherol. The only advantage of the synthetic vitamin is that there is no limit to the size of the dose which can be given, but there is no evidence that large amounts are ever needed, and some evidence that they are injurious (p. 767). Further, the synthetic vitamin contains none of the other tocopherols which may be of value, nor any of the vitamin B complex and other components of wheat germ which clinical results suggest are necessary for the proper utilization of vitamin E by the body. Vitamin E should not be injected (p. 714).

Further, the possible destruction of synthetic vitamin E in the stomach by incipiently rancid fats, still present from the last meal, cannot be

ignored (p. 729). The injurious effects of mild and unnoticed rancidity in the diet needs emphasizing most in relation to the synthetic vitamin because it is not protected by the anti-oxidants present in wheat germ.

Wheat Germ Oil. Wheat germ oil and its concentrates contain alpha-tocopherol, the other tocopherols, and are possibly more resistant than the synthetic vitamin to destruction by rancid fats during digestion (p. 729). They are thus more satisfactory than the synthetic vitamin, but they probably contain none of the vitamin B complex or other constituents of wheat germ. They also may lose their potency on keeping, a point of considerable importance, since between being prepared and consumed by the patient the oil, or the oil capsules, may have been kept in stock under bad conditions for many months.

Whole Wheat Germ. This is undoubtedly the best method of giving vitamin E. All the tocopherols are present and also the vitamin B complex and many other valuable substances. The anti-oxidants in the germ give considerable protection to vitamin E against destruction by rancid fats during digestion. Further, the germ keeps excellently if a reliable preparation is used: germ obtained from mills, however, may become rancid in a couple of days. It is difficult to understand why wheat germ oil should ever be given in preference to whole wheat germ. It is certain the oil cannot contain anything not present in the parent germ, and it is equally certain that much of value may be lost. Is, indeed, without doubt lost in as far as the vitamin B complex and minerals are concerned.

The trace elements, whose great importance in relation to the vitamins is only slowly being realized, should also be remembered when considering the value of wheat germ since it is one of their richest sources, notably for zinc [167] and manganese [168]. The trace elements are intimately connected with the proper use by the body of the vitamins [169, 170, 171] and so should be supplied when treating deficiency diseases, especially as the distribution of the trace elements in foods largely follows that of the vitamins, so that when there is a deficiency of the latter there is also most probably a deficiency of the former [167, 172]. The use of whole wheat germ ensures that the value of the vitamins it contains is not limited by the absence of those minerals which are essential for the full physiological activity of the vitamins and which are absent in purified and synthetic vitamin preparations.

Rarely children refuse to take wheat germ: wheat germ oil and the vitamin B complex should then be given instead [10, 104], and this combination may be used to give children a short rest from wheat germ during prolonged treatment.

Bicknell finds that wheat germ very occasionally causes an urticarial rash in children who are being treated for muscular dystrophy. It appears, like other forms of urticaria, most often after the child has had a hot bath or is warm in bed. After a few months it disappears; in the meantime it is best treated with the usual lotions and not with drugs. Donovan [108] has also seen a similar rash in a boy with muscular dystrophy who was taking wheat germ. Stone [104] using wheat germ oil for children with muscular dystrophy, and Shute [7] using it for adults with sterility or threatened abortions have reported occasional skin rashes. Newman [154]

using wheat germ and wheat germ oil caused a rash resembling lichen planus. Apart from these rare effects wheat germ and wheat germ oil are well tolerated, and do not cause any digestive disturbances.

Vitamin E should not be given close to a meal which has contained any food which may be on the verge of rancidity. In children cod liver oil, and cod liver oil concentrates, should be given at different meals to the wheat germ. All foods which are not fresh and all cheese should be avoided.

AMOUNTS OF VITAMIN E IN FOODS

In the following table—which is chiefly derived from that of Emmerie and Engel [21]—all the values for vitamin E are based on chemical estimations since no accurate biological estimations have been performed, owing to the enormous labour which these involve. This means that the values are probably, from the biological point of view, too high—possibly much too high—because in most of these chemical estimations (p. 712) no distinction has been made between the biologically active and the relatively biologically inert tocopherols (p. 709) which, in inconstant and unknown proportions, together comprise the vitamin E content of foods.

Food.	"Vitamin E" (see above) in mg. per 100 gm. or roughly $3\frac{1}{2}$ ounces.
<i>Cereals :</i>	
Barley	3.2-5.2
"Bemax"	24.5-35.0
Bread, "Brown"	2.1
"White"	1.4
Groats	1.2
rolled	1.5
Maize	10.0
Oats	2.1
rolled	2.0
Rice (½ polished)	0.4
Rye	2.2-3.5
Wheat Flour, 70% Extraction	1.7
82% Extraction	2.7
Germ	27.0 (70% α -tocopherol)
Whole Grain	1.2-3.4 (68.5% α -tocopherol)
<i>Dairy Produce :</i>	
Butter	2.1-3.3
Cheese, 20% Fat	0.6
10% Fat	0.3
Eggs, Boiled	3.0
Milk, 2.5% Fat	0.02 (see p. 739)
Pasteurized	? None [185]
Powder	0.5 (but see p. 739)
Powder (Skim)	0.05 (but see p. 739)
<i>Meat :</i>	See p. 714.

Food.	"Vitamin E" (see above) in mg. per 100 gm. or roughly $3\frac{1}{4}$ ounces.
<i>Oils and Fats :</i>	
Arachis or Peanut Oil	26-36 (62.5% α -tocopherol)
Beechnut Oil	100
Cocoa Fat	12.5
Coconut Oil	8.0
Cod Liver Oil	0 (but see Fish Liver Oils, p. 717)
Corn Oil	104-110 (5.8% α -tocopherol, Chiefly γ -tocopherol)
Cottonseed Oil, Refined	88-92 (50-75% α -tocopherol, 25-50% γ -tocopherol)
Fish Liver Oils	See p. 717.
Lard	0
Linseed Oil	23
Maize Oil	250
Olive Oil	8.0-8.0
Palm Oil (Red)	110
Sesame Oil	5.0
Soya Oil	92-120 (10% α -tocopherol)
Wheat Germ Oil, Crude	150-420
Medicinal	320
<i>Vegetables :</i>	
Beans, Kidney	1.2 } ? Low α -tocopherol
White	4.0 } content
Beetroot	0.2
Brussels Sprouts	1.7
Cabbage, Red	0.2
White	0.7
Carrots	1.5
Celery	2.6
Endive	2.0
Belgium	0.2
Kale	8.0
Leek	1.9
Lettuce	0.6 (but see p. 738)
Onions	0.2
Parsley	5.5
Peas, Green	5.4-6.4 } ? Low α -tocopherol
Grey	8.0 } content
Potatoes, Boiled	0.1
Radish, Black	0.04
Scorzonera	0.6
Spinach	1.7
Turnip	0.02
<i>Various :</i>	
Cocoa Powder	8.1

HUMAN REQUIREMENTS OF VITAMIN E

Nothing is accurately known of the human requirements of vitamin E. The amounts which are taken by people in good health are unknown because of the difficulties of estimating it in food. All that can be said is that symptoms of a deficiency, such as abortion, poor muscular development in infants and their increased growth with vitamin E, the reduction by vitamin E of the creatinuria of children, and muscular dystrophy are not uncommon. This, taken in conjunction with the decrease of vitamin E in modern diets caused by the introduction of white flour, makes it probable that the average diet is on the edge of a deficiency.

From analogy with the other vitamins, and from animal experiments, a great difference in individual requirements would be expected. This difference may be based on a personal idiosyncrasy, inherited or acquired, or on some morbid condition of the digestive tract hindering absorption. Infections may increase the need for vitamin E, and also, as Einarson and Ringsted [6] suggested, so weaken some systems of the body that they break down under the strain of a mild deficiency which in health would have been surmounted.

DISEASES DUE TO A DEFICIENCY OF VITAMIN E ABORTION AND OTHER SEXUAL DISORDERS

The tendency for the birthrate to decline among industrial or "civilized" communities is a serious problem. There are probably many causes for this among which a deficiency of vitamin E has often been considered of importance. Drummond and Wilbrahams in their book "The Englishman's Food" have drawn attention to the possible relationship between our falling fertility and the fall in our consumption of vitamin E with our increasing consumption of white flour from which the wheat germ has been removed. Maurice [106] has pointed out that the agricultural labourer of to-day has apparently a better diet than he did fifty years ago, but he eats white flour where he used to eat stone ground, and his family is smaller. To explain this low fertility by the spread of contraception ignores that it and white flour are both benefits of civilization. It also ignores that contraception is not likely to be efficiently used by most labourers.

Hogbin [107] mentions reports that the spread of refined foods low in vitamin E has been one of the causes of the falling fertility in some Pacific Islands, while Young [18] suggests that racial fertility is connected with diet, and that though the number of abortions due to a faulty diet is unknown, "we may, however, safely assume that it constitutes a considerable proportion of spontaneous cases." The importance of abortion, as apart from a decline in fertility, is shown by the fact that in England and America one-fifth of all pregnancies end in spontaneous abortion [112], while in France abortions from all causes are believed to be higher than the birth rate [108].

The average level of vitamin E in the blood, according to Varangot [250], during the first four months of pregnancy is 1.25 mg. per 100 c.c.,

during the next three months 1.65 mg. and during the last two months 1.82 mg. After delivery the level rapidly falls to normal.

Recurrent Abortion. Vitamin E has never gained much popularity with gynaecologists in the treatment of recurrent abortion. Browne [7, 109], for instance, has pointed out that as good results are obtained with endocrine preparations, simple rules of conduct, or indeed with no treatment save placebos. This rather hopeless attitude towards the problem may be criticized firstly on the grounds that Bacharach's [110] statistical examination of the published clinical results show quite definitely that vitamin E is of value in recurrent abortions. Secondly, because one form of treatment gives good results is no reason for doubting the value of another. In those women who tend to abort there may well be several mildly injurious factors—endocrine, dietetic, psychological—which added together terminate the pregnancy. The removal of any one will enable the pregnancy to proceed.

Vøgt-Møller [8] in 1931 first used wheat germ oil in the treatment of habitual abortion. He only reported two cases: treatment was successful in both though one woman had previously had four successive abortions and the other five. In the next five years he treated seventy-two women with no demonstrable reason for their recurrent abortions with 8 grams of wheat germ oil daily. Fifty-five living children were born, all of whom were well developed. In 1939 he summed up his own experiences and reports in twelve other papers. "Since 1936 my evidence from such cases has increased considerably. Taken as a whole, favourable results were obtained in about eighty per cent. of cases. Many investigations have confirmed my observations. The records of treated cases of habitual abortion now amount to some hundreds with a mean value of seventy-five to eighty per cent. favourable results" [7].

Currie [7] in 1939 reported his results from giving about 6 mg. of tocopherol daily in the form of concentrated wheat germ oil. The length of treatment varied from three to thirty-two weeks and was often not begun until the middle weeks of pregnancy. Treatment with luteinizing hormone did not give such good results. In all, eighty-one women were treated who had had two hundred and seventy-four previous pregnancies of which only forty-seven had gone to term. With treatment the next eighty-one pregnancies resulted in sixty-two viable infants being born. Isles [7] using the same dose of vitamin E as Currie treated eight women, who had had only five successful pregnancies out of twenty-nine, with complete success, eight viable infants being born.

Watson [111] had successful results in twenty-one of twenty-eight women who had had two or more abortions, and in eight of nine who had had one previous abortion. Lubin and Waltman's figures [152], from giving 8 mg. of synthetic vitamin E daily, were respectively five out of seven and eight out of ten.

Malpas [112], however, criticized the value of wheat germ oil on the grounds that it gave no better results than those which would have been expected had no treatment been given. From his own investigations, and those of others, he concluded that eighteen per cent. of all pregnancies end in abortion, and of these abortions only one is due to recurrent causes.

From this he deduced that in one hundred pregnant women treatment could at best only lead to eighty-three live births and better results than this proved too much. But why he should assume that lack of vitamin E could only cause recurrent abortions and not contribute to the seventeen per cent. of non-recurrent abortions is obscure. Many reasons might lead to a transitory deficiency of vitamin E which only was present during one conception, and so treatment with vitamin E might well lead to more than eighty-three per cent. of live births. In fact his figures suggest, if anything, that vitamin E may be one of the causes for so many women aborting once for no obvious reason. However, going on the assumption that seventeen per cent. of all pregnancies must end in abortion, he states that the expectation of living children in untreated cases of recurrent abortion is: after two successive abortions sixty-two per cent., after three twenty-seven per cent., and after four six per cent.

Bacharach [110] has examined the figures of Malpas and applied them in a slightly modified form to the published results of the treatment of habitual abortion. He points out that treatment has given far better results than would be expected from Malpas's figures were it valueless. In fact the probability of chance alone accounting for the apparent success of treatment is: after four or more consecutive abortions 1 in 10^{40} , after three or more 1 in 10^{10} , after two or more 1 in 200. This analysis of Bacharach's appears definitely to confirm the value of wheat germ oil in the treatment of recurrent abortions.

Threatened Abortion and Toxæmias of Pregnancy. Shute [118] observed that when rats became sterile from lack of vitamin E the resistance of their blood serum to proteolysis was increased. Treatment with vitamin E removed this antiproteolytic factor from the blood. He then investigated women who were threatening to abort and found that their serum was similar to that of vitamin E deficient rats in its resistance to proteolysis. Giving these women vitamin E returned the blood serum to normal.

Work by Jeffcoate [117], Shute [114], and others suggests that this antiproteolytic factor is of an œstrone like nature. Its presence hinders the proteolytic erosion of the placental villi into the uterine wall. This in turn leads to a weak attachment of the placenta and so to termination of the pregnancy.

From all these observations Shute [118] was lead to believe that "... vitamin E and œstrin, or a substance very much like it, exist in a sort of equilibrium during pregnancy. If there is too much of the œstrin-like substance the pregnancy is interrupted. An excess of vitamin E appears to have no effect on the pregnancy." Therefore in cases of threatened abortion with premature partial separation of the placenta and a high antiproteolytic factor in the blood vitamin E should be of value in treatment. Shute has treated a considerable number of such cases with wheat germ oil, and claims in one series success in sixty-eight per cent. of one hundred and eighteen cases [7]. Young [18] also found wheat germ oil of value in cases of threatened abortion, and so have others using synthetic vitamin E [152].

An objection raised to preventing threatened abortion occurring is

that it may be a sign of an abnormal foetus which would be better dead. But a study of all the available reports shows that only five out of eighty-nine children born after a threatened abortion had congenital anomalies [7].

Shute [7] emphasizes that women should have their blood examined for the antiproteolytic factor early in pregnancy. If it is high they will require wheat germ oil throughout the whole pregnancy. If this is not continued or is given in inadequate amounts toxæmia and premature placental detachment will occur later in the pregnancy, such as was observed by Young [18], who thought vitamin E given during pregnancy might unearth a later toxæmia.

Pregnancy toxæmias may be divided, according to Shute [115], into those produced by too little vitamin E and those produced by too little oestrogen. In the former type there is a raised blood pressure, oedema and albuminuria, and premature detachment of the placenta which gives rise to the name "hæmorrhagic toxæmia." This type responds to treatment with vitamin E. The second type is the true eclamptic with low oestrogens, and must on no account be given vitamin E, as this further depresses the oestrogens and so causes convulsions. Instead oestrogen therapy is required, though too much will convert the eclamptic to the hæmorrhagic type.

The amount of wheat germ oil advocated by Shute [7] is large and should be supplemented with vitamin B. An initial dose of $1\frac{1}{2}$ ounces of fresh oil are given, and then 1-2 drachms daily till the end of pregnancy. More may be needed to control the symptoms, especially towards the end of pregnancy and in patients with hypothyroidism, who tend to have high blood oestrogens. In the summer the higher content of vitamin E in the food decreases the amount of oil required.

Krieger [118], however, after very careful work could not confirm the presence of Shute's antiproteolytic factor, in the blood serum of cases of abortion, premature labour, accidental hæmorrhage or normal pregnancy. Cuthbertson and Drummond [118], using a slight modification of Shute's test [119] for the susceptibility of blood serum to proteolysis, failed to find any difference in the blood of rats reared on vitamin E deficient diets, normal diets, or diets rich in vitamin E. These authors also criticize the biochemical theory of the test. Drummond, Noble, and Wright [54] hold that Shute is wrong in believing that lack of vitamin E causes an excess of oestrogens in the blood, chiefly because if this were so lack of vitamin E would produce symptoms of an oestrogenic excess in animals, such as failure to conceive, irregular oestrus, and testicular changes unlike those observed from a deficiency of vitamin E. Shute [116] has answered these objections to his test and theory by saying his test was incorrectly carried out, and that the presence of small amounts of oestrogens in the blood of animals need not have the effects Drummond and his collaborators mentioned. In our opinion Shute's test is valueless.

Progesterone combined with vitamin E has been used, chiefly by German gynaecologists, for the treatment of threatened abortion. Winkler [240] reports that 80 mg. of alpha-tocopherol daily raises the low urinary excretion of pregnandiol which he has observed in cases of threatened abortion. He and Bach [241] advise giving progesterone with the vitamin E during the first five days of treatment in order to tide over the period

before the vitamin has had time to stimulate the corpus luteum. In seventy-four untreated cases there were twenty-four abortions, in twenty-seven cases treated with progesterone there were five abortions, in ten cases treated with 80 mg. of alpha-tocopherol there was one abortion, and in sixteen cases treated with both the hormone and the vitamin there were two abortions. Schäfer [242] successfully treated forty-five out of fifty-three cases with vitamin E alone or combined with progesterone. He does not state what dose of vitamin was given.

Primary Sterility. In both men and women primary sterility is probably not cured by wheat germ oil. This is not surprising since deprivation of vitamin E causes an irreversible testicular change in male animals, while in female animals vitamin E is not necessary for conception (pp. 720, 721). Schäfer [242] failed to cure primary sterility in women, but Shute [7] claims to have cured sterility in men. Mason [85] thinks that some human testes show changes due to a deficiency of vitamin E.

Lactation. Muller [120] reports that some human milk will only prevent sterility in rats (on a vitamin E deficient diet) if the mothers are given supplements of vitamin E.

Bennholdt-Thomsen [121] found that neither the fat content nor quantity of human milk is affected by extra vitamin E. Shute [7] claims that in a few cases wheat germ oil in doses of 2 ounces will cure defective lactation.

Menstrual Conditions. Amenorrhœa, dysmenorrhœa, menorrhagia and climacteric conditions are not affected by vitamin E [7, 248] with the possible exception of vaginitis and senile vulvo-vaginitis [7] and pruritus vulvæ [248].

Hain and Sym [248] report the relief or abolition of menopausal flushes, within three to eight weeks, by wheat germ oil containing the equivalent of 6 mg. of alpha-tocopherol daily. Only four cases were treated.

MUSCULAR DYSTROPHY

(PSEUDOHYPERTROPHIC MUSCULAR DYSTROPHY: PROGRESSIVE MUSCULAR DYSTROPHY: PRIMARY MYOPATHY)

Bicknell [9] in 1940 first produced clinical evidence which suggested that muscular dystrophy might be a deficiency disease caused by lack of vitamin E. Stone [10, 104] in the same and the following year published more definite results than Bicknell's, and since then a few further cases have been treated by other workers with success. But it is now clear from the clinical reports discussed on p. 758 that human muscular dystrophy is not, at least in the majority of cases, a simple deficiency disease identical with that caused in animals by lack of vitamin E.

The exact cause of the disease, if indeed it is one disease and not several diseases converging on one final common path, is unknown. A very full clinical account is given below because it seems probable that lack of vitamin E is a part of the cause, though in many cases not the whole

cause, of the depraved muscular metabolism. If the muscles degenerate because they have some obscure difficulty in utilizing vitamin E, then treatment with the vitamin alone will, in some cases, overcome this difficulty by providing a luxurious supply of the vitamin. On the other hand many cases will only respond to vitamin E when the fundamental metabolic error is also corrected. There is some tentative experimental work which suggests that the metabolism of vitamin E may be dependent on endocrine factors (p. 722) or on phospholipoid [246] or lipoid (p. 734) metabolism. There is also the common clinical impression that there is an abnormal endocrine or more particularly an abnormal pituitary background in cases of muscular dystrophy. Whatever is the whole answer it will only be found by using the results of animal research to correct whatever subtle dysfunctions are recognized from further careful clinical observation of the less obvious abnormalities found in muscular dystrophy.

Muscular dystrophy was first separated from other forms of paralysis by Duchenne of Boulogne, in 1861. The three types of muscular dystrophy generally recognized are the common pseudohypertrophic type found in children, which was first described fully by Duchenne [122] in 1868; the juvenile or scapulo-humeral form of Erb first recognized by him [123] in 1884; and the facio-scapulo-humeral type which bears the name of Londouzy and Dejerine [123] from their account of it in 1884. Intermediate forms are seen, and it must be stressed that all forms of dystrophy are only different manifestations of the same disease. The part which heredity plays has been exhaustively studied by Bell [123].

Pseudohypertrophic Muscular Dystrophy. This is the most common form of dystrophy seen in England, though it appears to be less common than the others in the United States judging by the relative numbers reported in recent papers. It occurs in all European countries and also in India, Ceylon and Japan, and there seems no reason to suppose any race is exempt.

Boys are affected far more often than girls. In the latter the condition tends to start later, often at the age of puberty, and to progress more slowly (Fig. 186).

A familial tendency is very marked, so that it is common to find in one family two or three brothers in different stages of the disease. Generally the girls are spared even when their brothers are not, and older boys seldom develop the disease after their younger brothers have done so. It is, in fact, the boys born after the one who develops dystrophy who require careful watching. Heredity appears to play no part in the disease, possibly because those affected have in the past died before the age of marriage.

The mothers of boys with muscular dystrophy, especially when the condition has apparently been present from birth, often give a history suggesting that during pregnancy and lactation they themselves were deficient in vitamin E. Thus it is not uncommon to find that before the dystrophic infant was born there were recurrent abortions, and recently two mothers attributed the dystrophy of sons born in the middle of long and normal families to starvation during these particular pregnancies. Prolonged nausea and vomiting has also been mentioned by mothers as a

possible reason. The number of cases investigated, however, is too small to make these observations more than suggestive.

The age of onset is commonly between five and ten, but many children give a history of crawling late and never walking properly, while others, especially girls, may show the first symptoms at puberty or even

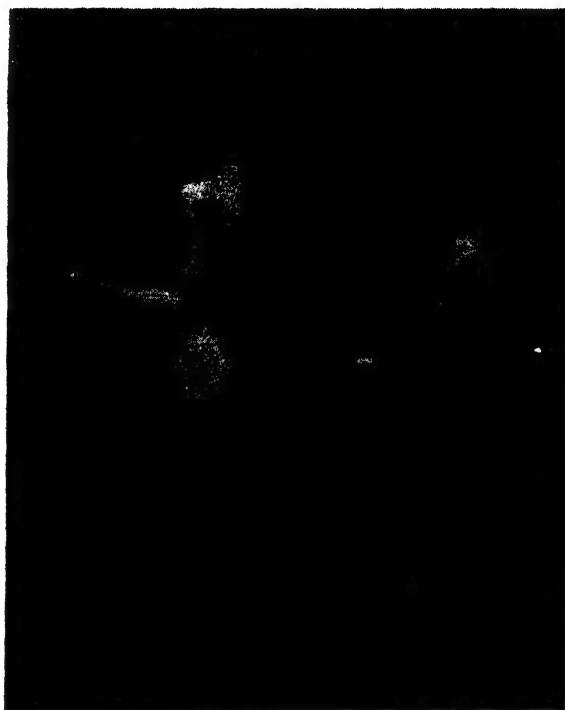


FIG. 186. Two English brothers, aged eight and six, with pseudohypertrophic muscular dystrophy. An elder brother is more seriously affected. Note the winging of the scapulae; the weakness of the shoulder girdles preventing the arms from being raised further; the low lumbar lordosis and the pseudohypertrophy of the calves and thighs.

later. In these late cases the condition tends to be less typical and to progress more slowly.

Infections often appear to unmask or emphasize the weakness so that the disease is ascribed to such complaints as scarlet fever or pneumonia.

The important changes occurring during the disease are increasing muscular weakness, increasing contractures, and a rare primary osseous atrophy.

Muscular Weakness. The picture of the disease is unique. It is that of a child fighting in an unsympathetic world against an increasing but unrecognized weakness of his legs. It is often surprising how weak the child becomes before he is taken to his doctor. Boys at school may be

blamed for months for being lazy and clumsy before any medical advice is sought. Usually the earliest symptoms are slowness in running, frequent falls so that the knees are never free from bruises, and hesitancy about going up, and especially down, stairs. Careful use is made of the banisters, and the children go down one foot at a time. In some children the frequent falls cause fear of slippery floors and loose mats.

If the weakness first shows itself in the lumbar muscles, and those joining the pelvis to the thighs, the typical low lumbar lordosis and protuberant stomach will appear. The waddling wide based gait will also be seen as the child dips from side to side in his painful efforts to overcome his inability to lift up his legs by swinging them outward and forward with each step. The weak dorsiflexors, evertors and external rotators of the feet cause the child to trip over his own dragging turned-in toes.

When the child falls down he has difficulty in getting up again, so that he "climbs up himself." This way of getting up is often thought to be pathognomonic of muscular dystrophy, but it is displayed in all cases where the muscles of the lower trunk and legs are weak. If the child is laid on his back and told to get up he first rolls over on to his stomach. Then he gets into a crawling position and slowly walks his hands backward toward his toes at the same time trying to straighten his knees and hips. The latter, however, he cannot do without the help given by his arms as he walks his hands up his legs (Fig. 191).

The shoulder girdles and upper arms are mostly affected long before the forearms, though after the legs. This leads to winging of the scapulæ and an inability to raise the arms above the head. Children whose shoulder girdles are weak are described as "slipping through" the hands of anyone trying to lift them by holding them under the arms. The flexors and extensors of the neck are often preserved to the end.

The face is commonly spared in this form of dystrophy, but the eyes in many cases appear to be unduly prominent, or to show more of the sclerotics than usual, giving a fleeting impression of hyperthyroidism. During sleep the eyes often remain open, the gruesome look of the face being most distressing to the mother.

The involvement of the muscles of respiration is generally late, but when it occurs is extremely serious, since the inability to cough turns a trivial bronchitis to a rapidly fatal pneumonia.

Swallowing is never, or very rarely, impeded, and the sphincters of the bowel and bladder always remain normal.

The nervous system is never affected, but the tendon reflexes are often lost early in the disease from the loss of tone in the muscles.

Irony is better shown in pseudohypertrophic dystrophy than in any other disease, since while the legs grow weaker their muscles tend to grow larger. This pseudohypertrophy often causes mothers to take a pathetic pride in their son's fine calves, and is also the reason why they realize so late that the legs are weak. Though the calves are most frequently, almost constantly, affected, any other muscles may enlarge. The extensors of the knees commonly undergo pseudo-hypertrophy at some stage, though the enlargement is often localized to one part of the quadriceps, or may

be so circumscribed that it is only obvious when the muscle contracts causing a sudden ball of muscle to rise up. This "ball" enlargement is also often seen in the muscles of the upper arm, the biceps, triceps, and less often the deltoids, giving the impression of hard but minute bunched up muscles, rather as if a pygmy boxer had given his muscles to a giant. Where the whole muscle is enlarged, as in the calves, the feel is typical. It has been described as woody or rubbery, but it is more like handling a piece of pickled pork. The lumbar muscles may be enlarged along their whole length, standing out each side of the spine. On the other hand any group of muscles may waste from the beginning of the disease, especially in girls (Fig. 187). In general, however, the muscles of the calves and thighs enlarge and the muscles of the upper arms and shoulder girdles waste.

Contractures. The importance of contractures in muscular dystrophy cannot be over emphasized. Indeed, they often appear to be as much a symptom of the disease as the weakness of the muscles themselves. They are generally stated to occur late in the disease when the child has become too weak to move, so that by sitting all day in a chair his thighs and knees and feet become largely fixed in one position. This, however, appears too simple an explanation. It is quite common to see children who have had their Achilles tendon lengthened, because it was too short, months or even years before there was any suspicion that they were dystrophic, and years before they ceased walking. On the other hand an occasional child

is seen with no trace of any contractures who has been confined to his chair for months with no massage or treatment of any kind. One is indeed forced to wonder whether the inconstant but often dominant contractures are due not to the primary muscular weakness, but to a primary change in connective tissue. Why this change is not found in all cases is obscure, but an analogy is seen in the rare primary involvement of the osseous system (p. 785). It is also reminiscent of a deficiency of the "anti-stiffness" factor which in some respects is similar to vitamin E (p. 798).

Probably so little notice has been taken of contractures because the

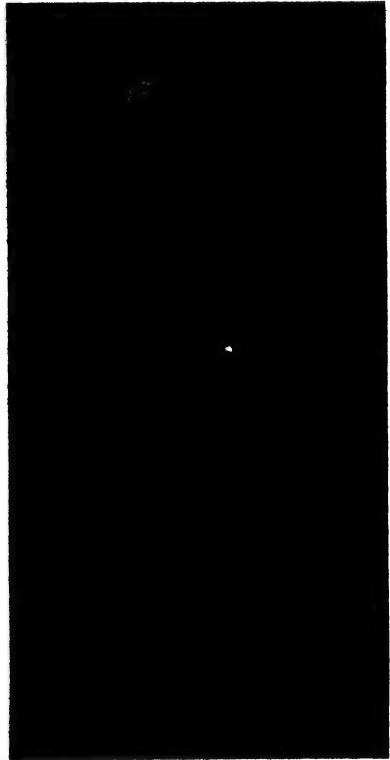


FIG. 187. English girl, aged thirteen, with muscular dystrophy. Note the low lumbar lordosis, and wasting of calves and thighs without any pseudohypertrophy. Sexual development is, if anything, precocious.

muscular weakness has been held to explain all the child's disabilities. Actually, however, the contractures are a major cause of his disabilities, especially through their effect on the feet and hips.

The feet, as has already been mentioned, may be drawn down by the contractures of the Achilles tendons, so that the child in the early stages tends to stand or walk on tip-toe, wearing out the toes of his shoes. As the deformity gets worse it is a very real hindrance to walking, even where the muscles of the legs are still moderately strong.

Contractures of the hips are seldom recognized, yet it is they as much, or more, than the weak pelvic muscles which lead to the grotesque lumbar lordosis of these children. To show that this is so it is only necessary to lie the child flat on his back, when he will be unable to keep his lumbar spine on the floor without flexing his thighs. If he is laid on his face flexing his knees will cause his buttocks to rise in the air, because the shortened muscles from his pelvis to his legs are thus stretched and so flex his body on his thighs. Kneeling in an upright position is also impossible for many children who can still stand, because in his position the shortened anterior thigh muscles pull the body out of the vertical as they are stretched by the flexed knees.

Contractures of the knees are seldom an early complication of muscular dystrophy, though of course when they occur they are an added difficulty in walking. Some limitation of movement at the elbows may occur early in the disease, but it causes little disability.

Osseous Atrophy. For many years it has been recognized that in a very few cases a primary osseous atrophy may occur at the same time as the primary muscular atrophy. Bramwell [124] believed that both these atrophies were manifestations of a change in the endocrine or sympathetic nervous system, and Maybarduck and Levine [125] also hold both conditions are independent processes of the same disease. The latter authors have reported one case of their own, and very fully reviewed the literature.

The bone atrophy has been described as an external concentric progressive atrophy.

The shafts of the long bones become progressively thinner, though the thickness of the cortex is not greatly reduced. Apparently as the outside of the cortex is removed the inside is reinforced with further bone, so that the slimness of the shaft chiefly occurs at the expense of the medullary cavity. The length of the bones remains normal. Cases are reported where the femora were no thicker than a little finger. The epiphyses of the bones are not decreased in size, so that the thin shaft connects two normally grown ends, giving a dumb-bell effect. The ends, however, may show considerable rarefaction. The flat bones of the pelvis and scapula may undergo some rarefaction as well as the bones of the feet and patellæ. Whether the small size of the winged shoulder blades seen in some cases is due to this atrophy or is secondary to the muscular weakness is uncertain. The fingers are thin and tapering.

Rarely the mandible is involved, developing a short vertical ramus and an obtuse angle, so that only the molars can be made to meet.

It appears definite that these bony changes are one of the primary results of the disease and are not merely a secondary disuse atrophy brought

about through the weak muscles. For no bone changes may be seen in very advanced cases, and when they do occur they may be present where the muscular power is still unimpaired. It is also significant that a case is recorded where there was both osseous atrophy and hypertrophy at the same time. Further, a boy with muscular dystrophy and osseous atrophy had a sister whose muscles were normal but whose bones showed the same typical atrophy as her brother's. Indeed it appears as if lack of vitamin E

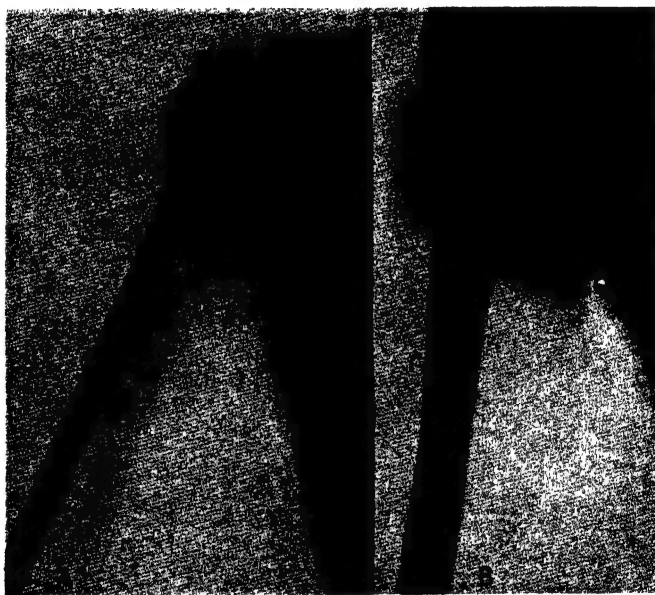


FIG. 188. X-ray A is of an Italian boy in America, aged sixteen, with muscular dystrophy and external concentric osseous atrophy (*see text*, p. 752). X-ray B is of a normal boy of the same age.

may sometimes only cause osseous atrophy, sparing the muscles completely. It is interesting to recall that vitamin E deficient rats may show osseous atrophy (p. 735).

Other Symptoms. Pituitary dysfunction is said by Bramwell [124], and hypothyroidism by Wechsler [126], to be a common complication of muscular dystrophy, as indeed it may be in vitamin E deficient rats (p. 724). Sexual development in boys often appears to be precocious, a point noticed both by Bicknell and by Armstrong [127]. Minot and Dodd [159] report that the urine of dystrophic boys contains oestrogens. Most children with muscular dystrophy have unusually good teeth. Mental precocity is common in muscular dystrophy, since the children are too much in the company of adults; added to this precocity is often a lively intelligence which never suffers any change.

Diagnosis. When the disease is fully established it is unlikely to be confused with any other. The diagnosis, however, is difficult in very

young children who have been late in walking and then not walked properly, or in children whose early symptoms all appear to be merely the result of short Achilles tendons. In such cases the estimation of the creatine and creatinine of the urine is of value, since the former is increased in muscular dystrophy and the latter diminished. Creatine tolerance is also lowered. These changes are found in all conditions where muscular function is impaired, so that they are really only of value

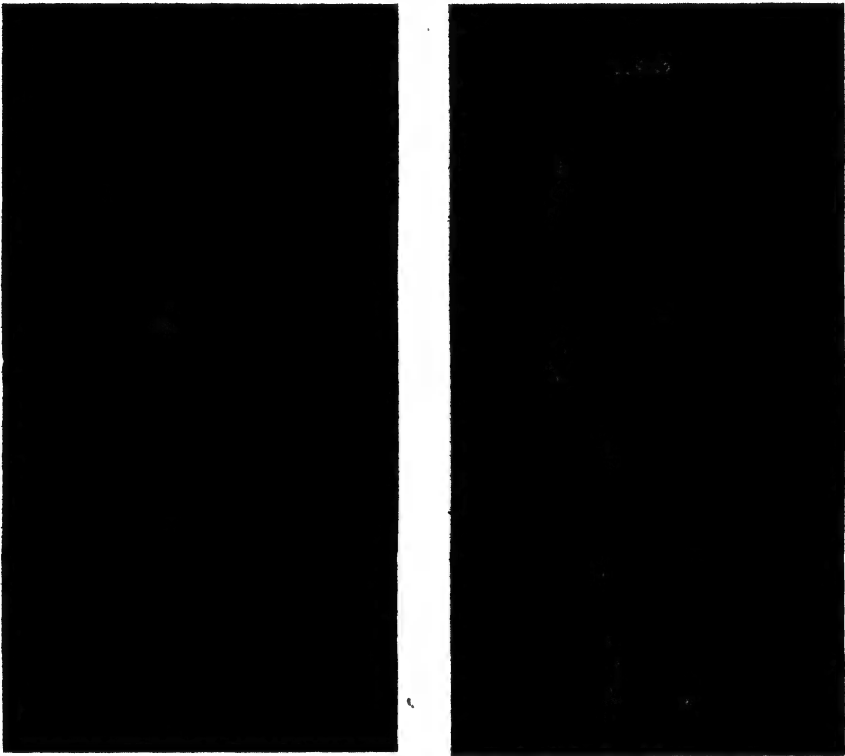


FIG. 189. English man, aged twenty-three, with pseudohypertrophic muscular dystrophy dating from early life, and progressing with unusual slowness. Note the pseudohypertrophy of the calves and possibly the triceps, the wasted thighs and buttocks and the low lumbar lordosis. Sexually he is very well developed.

in confirming that the child's symptoms are due to muscular weakness, where this is uncertain clinically. In children up to about the age of seven some creatine is normally present in the urine, but after this age it should disappear.

A biopsy on the most affected muscles will confirm a doubtful diagnosis (p. 727). The electrical reactions of the muscles are of no interest, remaining normal as long as any contractile muscle is left.

Course. Without treatment the child steadily gets weaker, generally dying from a respiratory infection somewhere in his teens. Even with

treatment if the chest expansion is bad when the child is first seen the outlook is very uncertain, as the most trivial bronchitis may end fatally in a few hours. This is due to the child being so weak that he cannot cough up any mucus, with the result that bronchial obstruction and pulmonary collapse may rapidly occur. The expansion of one side of the chest



FIG. 190. English woman, aged forty, with the facio-scapulo-humeral type of muscular dystrophy. There is some difficulty, not shown in the photograph, in closing the right eye and showing the teeth. Note the winged scapulae and weakness of the shoulder girdles preventing the arms from being fully raised.

may also be hindered by the extreme scoliosis which develops in children left all day slumped in a chair.

Some children become very fat : apparently this is not entirely due to lack of exercise, since they grow thinner with treatment even before they begin to move more freely. Most very advanced cases become extremely thin.

Facio-Scapulo-Humeral Type of Landouzy and Dejerine [128]. This form of muscular dystrophy is sometimes referred to as progressive muscular dystrophy. Intermediate forms, however, between it and the pseudo-hypertrophic type are common, and the same changes in the muscle

fibres occur in both. It seems, therefore, that the two diseases are fundamentally the same, though they differ in certain aspects.

Thus in the facio-scapulo-humeral type both sexes are equally involved and there is a strong hereditary tendency. The age of onset is also different, being commonly in later childhood, though infants and young adults may develop the disease. Progress is often so slow that the expectation of life is hardly diminished. One of the first cases to be described developed symptoms when he was twenty and died at sixty still pushing a hawker's barrow.

As the name implies it is the muscles of the face, shoulders and upper arms which are chiefly affected. The facial muscles first become weak causing the myopathic facies, with its prominent lips, transverse smile and inability to close the eyes or frown. The expression has been called stupid and sanctimonious [128]. With the involvement of the shoulder girdles the scapulae become winged, and as the weakness spreads to the upper arms many movements become impossible. Lastly, the thigh muscles become affected. Pseudohypertrophy is rare, and tends to be of the "ball" variety (p. 751).

The Scapulo-Humeral or Juvenile Type of Erb. This variety tends to occur at adolescence or in early adult life and often progresses slowly. The upper arms and shoulder girdles are affected first and many years may pass before the legs become weak, though progress is sometimes rapid.

Treatment of All Forms of Muscular Dystrophy. Before discussing the treatment of muscular dystrophy the reasons for considering it a deficiency disease in man, as it certainly is in animals, will be considered. That a deficiency of vitamin E is at least possible is shown partly by the poverty of our diet in vitamin E, and partly by its successful use in the treatment of recurrent and threatened abortions (pp. 738, 743).

Taylor [128] suggested that muscular dystrophy was not due to lack of vitamin E because it is a common disease in India where the diet largely consists of grain from which the germ, and so the vitamin E, has not been removed. Wright [129] and Bicknell [180], however, pointed out that the large consumption of rancid fat in India may destroy much of the vitamin in the food. Also it is probable that men, like animals, differ greatly in their requirements of vitamin E, in the same way they differ in their requirements of the fat soluble vitamins A and D.

The nutritional muscular dystrophy of guinea-pigs, rabbits, dogs and ducks which can be produced and cured by withholding or giving vitamin E has been described on p. 726. In all particulars it is similar to the muscular dystrophy of children. Both in animals and children histological examination of the muscles shows the same peculiar type of degeneration, not found in any other condition. Whether the brown pigmentation found in the uterine and voluntary muscles of vitamin E deficient animals may also occur in children, as was suggested to us by Cuthbertson, is not known. The nervous system in both is not involved, and in both the same changes in creatine metabolism occur. Both the primary osseous atrophy and endocrine changes which sometimes occur in dystrophic children appear to have their counterpart in animals (pp. 722, 735). Even the prominent eyes of pseudohypertrophic muscular

dystrophy have been noted in dystrophic rats (p. 735). Muscular dystrophy is most easily induced in young animals and most commonly occurs in young children. Infants and also animals may be born with the condition. In both without treatment there is a fatal termination, and the very slow downward progress of children can be matched in animals if their diet, like that of children, contains some, but insufficient, vitamin E.

The clinical results of treating muscular dystrophy with vitamin E furnish, of course, the only satisfactory proof of whether or no the condition is due to a vitamin E deficiency. Bicknell [9] treated his original cases and also many subsequent ones with whole wheat germ. His later results have been confusing: some children have continued to improve over the last four years, but in all these cases improvement has been very slow and treatment has always been carried out against a background of good food, good general health and good living conditions; other children have only improved for a few months before again deteriorating so that their initial progress may well have been due to the effects of encouragement and suggestion. The use of whole wheat germ diminishes the evidence that vitamin E itself causes this improvement, and certainly in Bicknell's hands synthetic alpha-tocopheryl acetate has had none but an evanescent effect.

Donovan [108] treated a boy with dystrophy with three-quarters of an ounce of wheat germ four times a day for six weeks with very marked success.

Dr. Armstrong, in a personal communication, says that his children on wheat germ have felt better, but biopsies on the muscles of two before and after six months' treatment have not shown any improvement. Professor Telford and Mr. Lilie have both had good preliminary results.

Stone [10, 104] in America has reported six children treated with 2 to 4 c.c. of fresh wheat germ oil daily. All improved, and one completely recovered. Progress was hastened by adding the vitamin B complex to the oil, thus giving a mixture very similar to wheat germ. Minot and Dodd [159], giving "large amounts" of vitamin E, improved two of five cases.

Investigations by Bicknell and Prescott on whether synthetic vitamin E reduced the creatinuria in six children with muscular dystrophy were interrupted by the bombing of London. Fleischman [181] has reported that giving 100 to 220 mgm. of alpha-tocopherol daily to two cases of muscular dystrophy had no effect on the creatine excretion. But he treated his cases for less than four weeks, a period which is far too short to show any clinical improvement, and so could hardly be expected to affect the creatinuria. The results of Fitzgerald and McArdle [182] also have no significance owing to the short period of their investigation. Harris [184] observed three dystrophic children for about nine months while they were taking up to 200 mg. of alpha-tocopherol daily by mouth. He found no change in the excretion of creatine and creatinine or in their physical condition. Minot and Frank [208] also failed to reduce the creatinuria in eight boys, five to nineteen years old, with varying doses of synthetic vitamin E or wheat germ oil. All these negative results are perplexing

since vitamin E immediately reduces the slight creatinuria of normal young children [164] and so should at least cause a slight fall in the total creatine excretion of dystrophic children. On the other hand Milhorat and his co-workers [188] treated two cases of dermatomyositis with wheat germ and observed, after only two weeks, a marked and progressive decrease in the creatinuria, accompanied by clinical improvement in the muscular condition.

American reports denying the value of vitamin E in muscular dystrophy have been made by Doyle and Merritt [184], Sheldon and others [185], Ferrebee, Klingman, and Franz [186], Pohl and Baethke [186] and Minot and Frank [203]. The first of these reports deals with a man of thirty-three who had had muscular dystrophy for ten years and a girl, aged eighteen, who had been affected for six years. The latter was only treated for three weeks. The former, however, over a period of five months became slightly worse, though he was given large amounts of vitamins A and D, the vitamin B complex and liver extract. He also received every day 2 drachms of wheat germ oil and 60 mg. of alpha-tocopherol by injection. The second report mentions eight cases of muscular dystrophy, aged 5, 19, 28, 28, 80, 34, 38, 58. Treatment was with either 100 mg. of alpha-tocopherol injected twice weekly, or 50 mg. given daily by mouth. In every case 3 tablespoonfuls of wheat germ oil were taken after each meal. The duration of the treatment is vague. Ferrebee and his collaborators [186] treated twenty cases, of whom thirteen were aged fifteen or less. Treatment was continual for from four to fifteen months. It consisted of the daily consumption by mouth of two tablespoonfuls of wheat germ, 110 to 190 mg. of alpha-tocopherol, and 10 to 30 mg. of vitamin B₆. Injections of 100 to 400 mg. of alpha-tocopherol were also given each week. Pohl and Baethke [186] gave a wide range of oral doses of all forms of the natural and synthetic vitamin to fifteen cases for nearly two years with negative results, and Minot and Frank [203], administering wheat germ oil or the synthetic vitamin by mouth, also failed to obtain any response in eight boys. In these boys the level of vitamin E in the blood was normal (p. 715) before treatment and rose after.

In Spain Alemany Soler [244] in 1943 reviewed the results of many workers, the great majority of whom failed to obtain any benefits from vitamin E, though a few reported cures. In England Fitzgerald and McArdle [182] have reported negative results in five children given either wheat germ, or wheat germ oil, or synthetic vitamin E. The details and duration of the treatment are rather obscure.

Two criticisms of all these negative results can be made. Firstly, adult cases, in whom the disease has lasted for many years, cannot be expected to improve as the greater part of their weakness must be due not to muscle fibres which are still degenerating but to fibres which have completely disappeared [186].

Secondly, many of the young cases whose muscles should have been capable of some regeneration had large, in most cases huge, doses of alpha-tocopherol. In fact, most of them were taking as much vitamin E as would be contained in a daily consumption of 50 lb. of wholemeal

bread. Such quantities would appear to be far beyond any physiological requirements, and to be thirty to forty times as large as those given to the cases of Bicknell [9], Stone [10, 104] and others (p. 757) which have improved. They are also much larger than the doses used in other muscular conditions with success (p. 765). It must also be remembered that Wechsler and others [157] report that in adults injections of 100 mg. of alpha-tocopherol actually cause a fall in the level of vitamin E in the blood. This means, if confirmed, that injecting the vitamin will increase its deficiency.

Nothing is known of where or how excess vitamin E is destroyed, but since it is mostly stored in the muscles it well may be that they not only utilize vitamin E but also destroy any excess. If this is so muscular metabolism might be overburdened by the effort of destroying large amounts. The stimulating effect of large quantities of vitamin E on phosphorus metabolism in muscle (p. 718) may also be overwhelming. Indeed, some of the American cases give the impression that they deteriorated faster when given massive doses of vitamin E.

There is some slight and indirect evidence that vitamin E can be toxic. The work of Weissberger and Harris [211], who showed that very large doses of vitamin E cause decalcification of the bones of rats, has been described on p. 718. Shute [116] has mentioned three cases where vitamin E caused convulsions during pregnancy toxæmias, and he also states that Barrie has seen the same effect in rats. Bicknell (p. 767) in three neurological cases found alpha-tocopherol toxic, the toxicity apparently being reduced by vitamin B. A personal communication from South America also mentions the toxicity of synthetic vitamin E in amyotrophic lateral sclerosis. That vitamin E may be toxic is not surprising in view of the toxicity of the fat soluble vitamins A and D. It is interesting to note that vitamin B decreases the toxicity of vitamin D (p. 698).

It is also germane to remember that there is some evidence that the amounts of the vitamins in the diet should bear some relationship to each other. Besides the possible importance, mentioned above, of vitamin B when vitamins D and E are taken in large amounts, there is also the relationship of vitamins A and E (p. 42). Jukes, in a personal letter, also gives an excellent example of the interdependence of the vitamins: "... if chicks are fed a diet deficient in riboflavin and pantothenic acid, dermatitis does not appear, but if riboflavin is added to the diet, acute symptoms of pantothenic acid deficiency become manifest. . . ." Bicknell is certain that wheat germ containing about 6 mg. of alpha-tocopherol and, of course, small amounts of other tocopherols, the vitamin B complex and other substances such as the trace elements (p. 740) is the best form of treatment. Stone [10, 104] emphasizes the importance of adding the vitamin B complex to wheat germ oil. He also draws attention to the possible importance of substances other than alpha-tocopherol in wheat germ oil.

Along such lines as these probably lies the explanation of why small amounts of wheat germ, or wheat germ oil and the vitamin B complex; may cause a slow and continuous improvement in cases of muscular dystrophy, while at best no lasting effect is gained by large amounts of

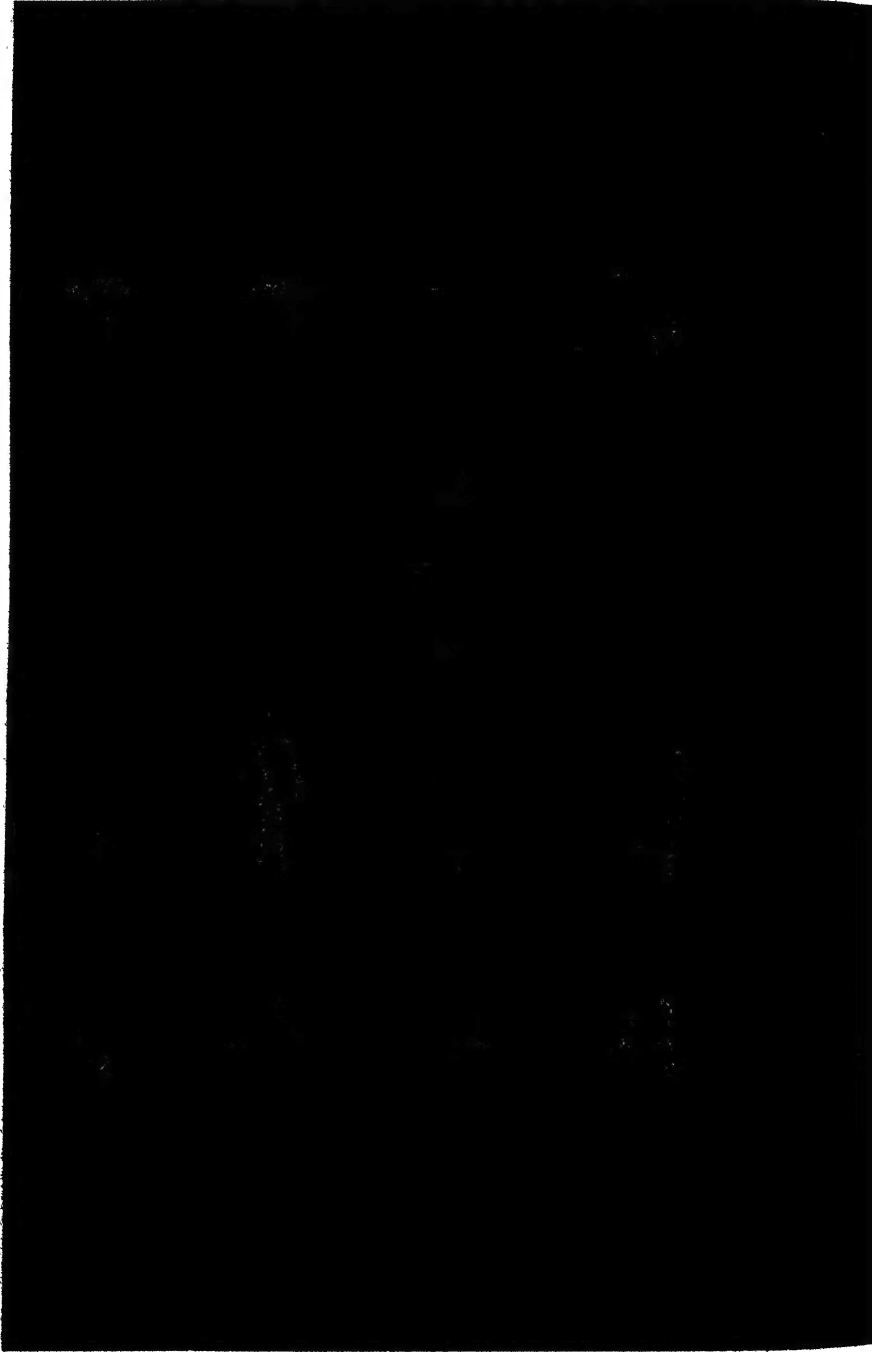


FIG. 191. Typical movements of an English child

medohypertrophic dystrophy. (See text, p. 750.)

alpha-tocopherol insufficiently balanced by other tocopherols, the vitamin B complex and the other constituents of wheat germ.

Form in which Vitamin E should be administered. From the above discussion and that on p. 740 it appears that the most satisfactory way of administering vitamin E is to give whole wheat germ and fresh foods rich in vitamin E (p. 739). About 1 ounce a day of wheat germ is ample. Most children prefer it taken like a "breakfast food" before breakfast, mixed with cold milk. It is essential that the wheat germ should not be stale or rancid.

Wheat germ oil and the vitamin B complex may be given to those unusual cases who dislike wheat germ. Apart from the greater expense of this form of treatment there is always the uncertainty as to how fresh is the wheat germ oil, and so how much of its vitamin E potency is retained. In England wheat germ oil, and wheat germ oil concentrates, are usually sold in capsule form, and are reported not to deteriorate. The unconcentrated oil is probably best. About 6 mgs. daily of alpha-tocopherol in this form should be sufficient. Stone [10, 104] gives about 2 to 4 c.c. of fresh wheat germ oil daily. Injections of wheat germ oil or synthetic vitamin E are not advised (p. 714).

Wheat germ, wheat germ oil, and synthetic vitamin E all may cause an irritating urticaria. This generally passes off in a few months.

Rancid fat destroys vitamin E, so that any food like rancid butter, sour milk, or cheese must be avoided (p. 729).

The diet should also contain as much food rich in vitamin E as possible, especially stone-ground bread, and green vegetables in large amounts. The former is well worth the trivial trouble of making it at home. Such a diet unsupplemented with any preparation of vitamin E is reported to have cured two children of muscular dystrophy and to have improved three others.

Liquid paraffin, or paraffin emulsions, should not be used as aperients, as they probably hinder the absorption of vitamin E (p. 718).

Bile salts should be given by mouth with the vitamin if there is any intestinal history suggesting that bile may be deficient, since it is necessary for proper absorption (p. 718).

Vitamin B₆ given by mouth or by injection, with or without vitamin E, has been found to have no value in muscular dystrophy by Bicknell and by others [182, 184, 186], so the claims of Antopol and Schotland [149] appear to be incorrect. In any case wheat germ contains vitamin B₆. Inositol may be of value [259, 260].

Vitamin C has been said to cure muscular dystrophy [188], but Bicknell noted no benefit to three cases taking 1,000 mg. daily by mouth for two months.

Duration of Treatment, Rate of Progress and Prognosis. Treatment must last for years rather than months. It cannot be hastened and may be retarded by increasing the amount of vitamin E mentioned above. Stone [104] has reported the complete cure in six months of a boy of eight who was a fairly advanced case and had been affected since infancy, and personal reports mention cures in two cases. Bicknell has treated two cases for four years and they have continued slowly, but steadily, to

improve. Both cases were very advanced when first seen. One, a boy of four, would fall about ten times while walking down the ward; now he can walk a mile normally. The other boy was eight when treatment started and was virtually bedridden and unable to touch his own ears. He now can walk several hundred yards and lift a heavy book over his head. But improvement is never dramatic, and parents must be made to understand this.

How far improvement may go it is yet too early to state. Muscles which appear to have completely vanished may in the course of months start to function. In the present state of our knowledge it appears reasonable to treat all children however hopeless they appear.

For children who are still walking the outlook would appear for some to be excellent, and even those who are chair-ridden have improved so much that it seems probable that with years of treatment they will recover sufficiently to earn their living in sedentary work.

The outlook is worse in children who have very poor chest expansion because here sudden death from mild bronchitis is common (p. 754).

Sepsis delays or stops progress, even if it is mild, such as carious teeth, sinusitis, otitis media, or ingrowing toe nails. It cannot be over-emphasized that sepsis must be treated.

Infectious diseases such as scarlet fever tend to make the child worse for a time, but, on the other hand, they may have no effect. Children should be got up as soon as possible after illnesses. Treatment with the sulphonamides has no adverse effect on the muscular condition, and has even been reported to be beneficial [260].

Treatment of Contractures, Massage and Exercise. Contractures in the majority of cases greatly increase the disability (p. 751). Almost constantly there is a shortening of the Achilles tendon which, added to the usual extreme weakness of the flexors and evertors of the feet, makes walking difficult. At the same time weak external rotators of the thighs may allow the feet to turn in so that the child trips up over them.

Adjustable light splints worn at night will help to prevent further shortening of the calf muscles and also will tend to passively lengthen them. Where splints are tolerated badly and the child sleeps on his back, pressure can be kept on the outer front of the sole by arranging that the feet press on a padded board pushed upward from the end of the bed. Raising the head of the bed slightly can also be used for pushing the feet against their support.

Very simple exercises should be given several times a day to make the child use the dorsiflexors and evertors of the feet and the rotators of the thighs. It is remarkable how a child who has stopped using a muscle will not begin to use it again as it grows stronger, unless he is taught to do so.

The contractures of the thighs and knees are partly checked if the child sleeps flat on his back, but it is not worth sacrificing sleep in trying to make children stay in a position they dislike. For at least an hour a day all children, especially those with contractures, should lie flat on their backs on the floor or table with only a small cushion under their heads. If the adductors of the thighs are so weak that the knees flop apart the

legs should be tied loosely together with a scarf. This rest is good for all children, and also tends to pull out the flexion contractures of the thighs and knees.

When sitting the feet should be drawn under the child to increase dorsiflexion, but sitting is always a very bad position because it favours contractures of the thighs and knees. Children instead should, as far as possible, recline so that the angle between their body and thighs is increased. When children are too weak to change their own position it should frequently be changed for them. Lateral curvature of the back must be avoided.

Gentle passive stretching of the contractures should be carried out several times a day. Laying the child on his back on a table so that his thighs hang unsupported over the end is an excellent method of stretching contractures of the thighs.

Boots with high heels and the outer side wedged may be used to help in walking. The boots must be light with flexible soles, and as the calf muscles stretch, the heels must be cut down. Spinal jackets and other forms of support must never be employed.

Surgical treatment of contractures is seldom necessary, being generally corrected by the above methods. In any case the longer an operation is postponed the stronger will be the muscles and the less the likelihood of a recurrence.

General light massage is valuable for children who can move but little. The great value of a masseuse, however, is to teach the child to use his muscles again as they grow stronger. Very simple, varied, and amusing exercises must be given which the child and his mother can do several times a day. Ideally every joint and muscle should be fully used every day. Faradism should not be employed.

Exercise is good as long as it is not forced. Children will not over-tire themselves when playing alone, and such games as "Red Indians" make them use combinations of muscles which are difficult with set exercises. A puppy is an excellent playfellow for a dystrophic child. Swimming or playing in the water is often possible when walking is not, because the body is supported by the water. On the other hand it is so important to avoid infections that public baths should never be used. If the child gets cold he will for a few hours be considerably weaker.

Glycine in the Treatment of Muscular Dystrophy. Armstrong [127] has given a thorough review of the use of glycine in the treatment of muscular dystrophy, and has reported its effects in eighteen cases of his own, many of whom were treated for more than three years. He also followed the effects of treatment with biopsies on muscles. It appears that glycine may give a temporary improvement in some cases, but it is certainly of no lasting value in males, and probably does not influence the ultimate course of the disease in females. The experimental work of Ni, which Armstrong mentions as supporting the use of glycine in dystrophy, is discussed on p. 729, where it will be seen that Ni himself now does not consider that his experiments showed that glycine itself influenced the nutritional dystrophy of animals. The transitory clinical benefit of glycine is probably due to its increasing the power of normal muscle [187].

FURTHER CLINICAL USES OF VITAMIN E

Wheat germ has very much the same position and importance as cod liver oil as a dietetic supplement, except that the constituents of wheat germ are often deficient in the diet throughout the year, while in the summer the vitamins of cod liver oil are generally plentifully supplied from other sources.

Development and Growth. Donovan [108] has reported a family where the son had muscular dystrophy; the daughter postural lordosis, a protuberant abdomen, winged scapulæ, absent knee jerks and muscular pains; the father a fixed lordosis, and the mother, though not using contraceptives, had had only two children in twelve years. Three ounces of wheat germ a day for six weeks made both parents feel better; the boy greatly improved; the girl's lordosis and muscular pains disappeared, and her knee jerks returned. Donovan suggests that postural lordosis in children, such as the girl's, may be a minor or arrested form of muscular dystrophy which is frequently not recognized. In later life it leads to a fixed lordosis, like that of the father.

Stone [104] treated a large group of children between the ages of one and five who were seen because of poor muscular development, late standing and walking, and inability to hold up their heads until they were two or three. Treatment with vitamin B had little effect, but rapid improvement occurred when 8 to 12 minims daily of fresh wheat germ oil were given as well. He believes that young children with muscular hypotonia are very mild cases of a vitamin E deficiency resulting from poor placental supplies.

In eleven of seventeen premature infants Widenbauer [141], using wheat germ oil, caused a rapid increase in weight after it had been stationary for some time. His results were carefully controlled, and appear important; they are supported by one Spanish case [141].

Amyotonia congenita, according to Stone [104], is the result of a very severe intra-uterine deficiency of vitamin E. Treatment with 2 to 4 c.c. of fresh wheat germ oil and the vitamin B complex gave excellent results. Bicknell [9] also reported improvement in one case treated with whole wheat germ. A girl of twenty given synthetic vitamin E is reported not to have improved [182].

Pink disease has been treated with wheat germ by Forsyth [188] in Australia. In his first paper he began by thinking that vitamin B₁ was the deficient vitamin, but it is interesting to see how in his next two papers his clinical results forced him to ascribe the successful results of using wheat germ more to its other vitamins, especially vitamin E, than to vitamin B.

Syphilitic interstitial keratitis is stated by Stone [247] to respond to wheat germ oil combined with riboflavin or the vitamin B complex. Ten cases are reported who all had previously had extensive anti-syphilitic treatment. Even in the old cases with marked opacities and scarring of the cornea complete recovery occurred after several months. The experimental justification for this therapy is discussed on p. 735.

Allergic conditions, according to Glaser and Dam [248], do not respond

to vitamin E in spite of the effect of lack of the vitamin in increasing capillary permeability (p. 784). Twenty-five cases of ragweed pollinosis were given 250 mg. of alpha-tocopherol acetate daily, but there was no improvement compared to twenty-five untreated cases. There was also no response in a few cases of asthma, urticaria and allergic rhinitis.

Leprous nerve and bone pains have been treated successfully by Cochrane, Paulraj and Salmond [139], who substituted whole wheat for rice in the diet. The results are impressive, and cannot be accounted for by an increased consumption of vitamin B₁. The authors suggest this form of treatment before operating on cases with a painful neuritis, though they point out that the leprosy itself is not affected. Vitamin A is reported to be of value for leprosy ulcers (p. 64).

Fibrositis. Steinberg obtained excellent results with wheat germ oil [148], or with alpha- or gamma-tocopherol [198], in primary fibrositis, but other forms showed little improvement. This is confirmed by Ingham [198]. Two cases of dermatomyositis improved on whole wheat germ [188].

Carcinoma and Leukæmia. Vøgt-Møller [7] gave four cases of gastric carcinoma and one child with acute lymphatic leukæmia vitamin E (? whole wheat germ) with negative results.

Debility. Spies and Vilter [140] treated fourteen adults who were complaining of extreme weakness, inability to work, poor appetite, insomnia, aches and cramps in the legs, and burning feet with a single injection of 500 mg. of alpha-tocopherol. In twenty-four hours twelve were so much better that they could do a day's work for the first time for months. After four weeks eight patients were still at work, but four required a further injection.

VITAMIN E IN THE TREATMENT OF NEUROLOGICAL CONDITIONS

The experimental work of Einarson and Ringsted (p. 781) raised high hopes that vitamin E would be of value in certain degenerations of the central nervous system—notably amyotrophic lateral sclerosis and tabes dorsalis. The weight of evidence, however, is now against this, though it must be admitted that the position is not yet clear. From the clinical results discussed below it appears that alpha-tocopherol used alone and in large amounts is at best of uncertain value in progressive muscular atrophy and amyotrophic lateral sclerosis. But there are too many reports of vitamin E having had a transitory or prolonged effect on nervous degeneration for them to be completely ignored.

The subject is extremely complex. There is firstly the experimental evidence of Einarson and Ringsted that once lack of vitamin E has initiated a nervous degeneration, the addition of vitamin E to the diet will not check further degeneration if this is at all advanced. Secondly, the experimental work of Wintrobe and his colleagues [144] has shown that a degeneration of the central nervous system in pigs can be produced by food deficiencies, though the deficient factors have not been identified. Thirdly, synthetic vitamin E may be toxic in large amounts and may require the vitamin B complex for its proper utilization by the body (p. 759). Fourthly, as has been pointed out by Wechsler [145], similar

nervous degenerations may, in different people, have different causes—thus some cases of amyotrophic lateral sclerosis may be due to a food deficiency, some to a vascular degeneration, and some to other causes. Lastly there is Davison's outstanding work, to be discussed later, which strongly suggests that vitamin E profoundly modifies pathological changes in the central nervous system, even if it cannot prevent them.

The impression left from the clinical reports is that we are still fumbling in the dark close to a solution of many of the nervous degenerations, but that until more light is thrown on the subject the patient's best chance is to be treated with natural vitamin E and the B complex, and a high intake of foods rich in the other vitamins. This does not necessarily imply that the degenerations are caused by a food deficiency, but it does



FIG. 192. Case I. Lack of visible demyelination in the pyramidal pathways of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 193. Myelin sheath stain.

mean that any deficiency which is hampering the nervous system in its recovery is removed.

Amyotrophic Lateral Sclerosis and Progressive Muscular Atrophy. Bicknell [9] in 1940 reported good results in two of four patients treated with whole wheat germ, but later cases treated with either wheat germ, or synthetic vitamin E have only shown, at best, a mild objective and subjective improvement for a few weeks before continuing to deteriorate. In three cases, and one reported from South America, synthetic vitamin E has caused extremely painful cramps or spasms of the legs, occurring chiefly at night. Two patients regulated their own dose of vitamin E by these cramps, which came and went as the dose was increased or diminished. In one case the pain was so severe that a fatal collapse was feared, though not more than 20 mg. of alpha-tocopherol was being taken. A case of disseminated sclerosis also developed cramps whenever the daily intake

rose about 9 mg. The vitamin B complex appeared to increase the tolerance for vitamin E in these cases. One patient who had marked euphoria gave up wheat germ oil because it changed his euphoria to depression.

Wechsler [173] has now reported sixty cases whom he has treated with synthetic vitamin E in 50 mg. doses daily either by mouth or injection. He also gives wheat germ oil, the vitamin B complex, foods rich in vitamin E, and bile salts to aid the absorption of the latter. Ten of his cases have shown varying degrees of improvement, two completely recovering. The longest period of treatment has been two years. His paper [145] should be read for an excellent discussion on the general problems and implications

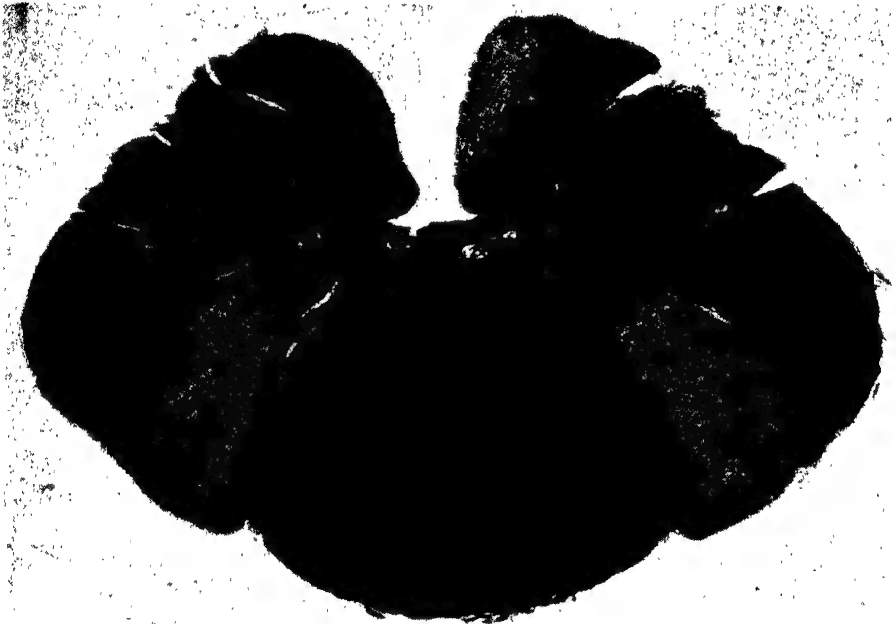


FIG. 193. From a non-treated case, showing extensive demyelination of the crossed pyramidal and left direct pyramidal tracts. Compare with Fig. 192. Myelin sheath stain.

raised by considering such cases are due to food deficiencies. Some of his cases improved or got worse as the vitamin was given or withheld [145]. He and others [157] report that in seventeen cases the average tocopherol content of the blood was 0.67 mg. per 100 c.c. compared to 0.96 mg. in twelve normal subjects, the level in the latter varying between 0.59 and 1.62 mg. Transitory clinical improvement with oral doses did not occur unless the level was raised by at least 0.4 mg., this being only achieved by doses of at least 200 mg. daily. Injections of vitamin E caused a fall in the level in the blood, which is analogous to the decrease in the level of vitamin A after it is injected.

Einarson, Ringsted, and their collaborators [150] state that with 90 mg. of alpha-tocopherol their results from eight cases "furnish some

AMYOTROPHIC LATERAL SCLEROSIS



FIG. 194. Case I. Insular myelin sheath destruction from a case treated with vitamin E. Compare with Fig. 195. Myelin sheath stain. $\times 240$.



FIG. 195. Extensive myelin sheath destruction from an untreated case. Compare with Fig. 194. Myelin sheath stain. $\times 240$.



FIG. 196. Case I. Slight disintegration and swelling of single myelin fibres in parts of the pyramidal tracts that appeared uninvolved, from a case of amyotrophic lateral sclerosis that received vitamin E. Compare with Figs. 194 and 195. Myelin sheath stain. $\times 480$.

THE VITAMINS IN MEDICINE

AMYOTROPHIC LATERAL SCLEROSIS

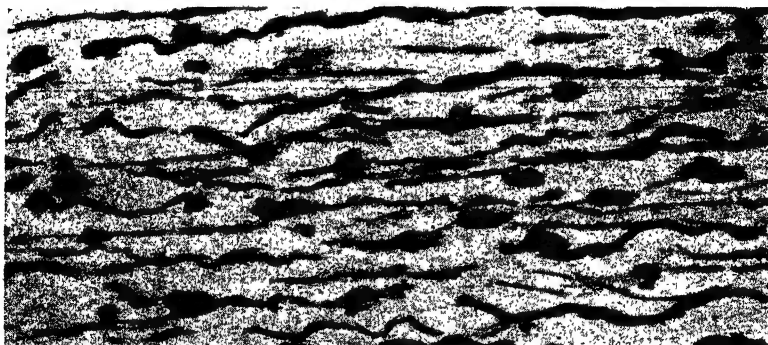


FIG. 197. Case I. Axis cylinders from the crossed pyramidal tract of a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 198 from a normal case, and Fig. 199 from an untreated case of amyotrophic lateral sclerosis. In Fig. 197 there is slight diminution in number, swelling and slight tortuosity of axis cylinders when compared with the normal in Fig. 198 and the severely diseased, fragmented and swollen axis cylinders of the untreated case of amyotrophic lateral sclerosis in Fig. 199. Bielschowsky stain. $\times 480$.



FIG. 198. From a normal control case, for comparison with Figs. 197 and 199. Bielschowsky stain. $\times 480$.



FIG. 199. From an untreated case of amyotrophic lateral sclerosis. For comparison with Figs. 197 and 198. Bielschowsky stain. $\times 480$.

basis for believing that vitamin E may have an effect at least on neurogenic muscular atrophy."

Meller [174] treated fourteen cases with 100 to 250 mg. of alpha-tocopherol daily by mouth or by injection for periods of three to seven

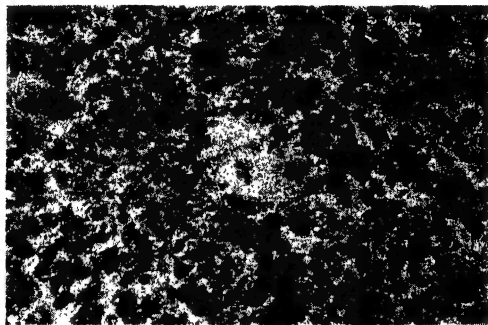


FIG. 200. Case I. Almost complete absence of fat in the pyramidal pathways from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 201 from an untreated case showing lipid deposits throughout and in the perivascular spaces. Sudan III stain. $\times 100$.

months: three recovered and seven improved. Rosenberger [175] relieved some of the symptoms in eight of nine patients with a diet rich in vitamin E, synthetic vitamin B₁ and 100 to 200 mg. of alpha-tocopherol given orally. His patients were observed for about a year. Donzallaz and Monnier [176] in Switzerland treated an interned Alsatian soldier fo



FIG. 201. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig. 200. Sudan III stain. $\times 100$.

three months with 80 mg. of alpha-tocopherol daily by mouth. There was a considerable degree of recovery, writing and other activities becoming possible. During a short period with no treatment his weakness returned. Gotten [177] reports good results in two patients, Viets [178] improvement in one patient among twenty-one, and Slaughter and Cleckley [178] one who completely recovered on 1 drachm of wheat germ oil daily.

AMYOTROPHIC LATERAL SCLEROSIS

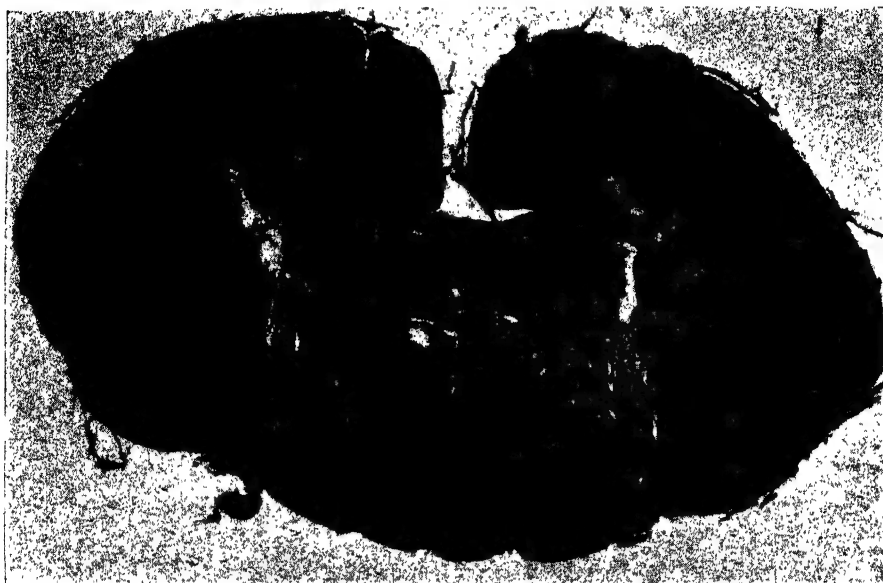


FIG. 202. Case I. Lack of gliosis in the pyramidal tracts from a case of amyotrophic lateral sclerosis treated with vitamin E. Compare with Fig. 203 from an untreated case of amyotrophic lateral sclerosis showing dense gliosis in the crossed pyramidal tracts. Holzer stain.

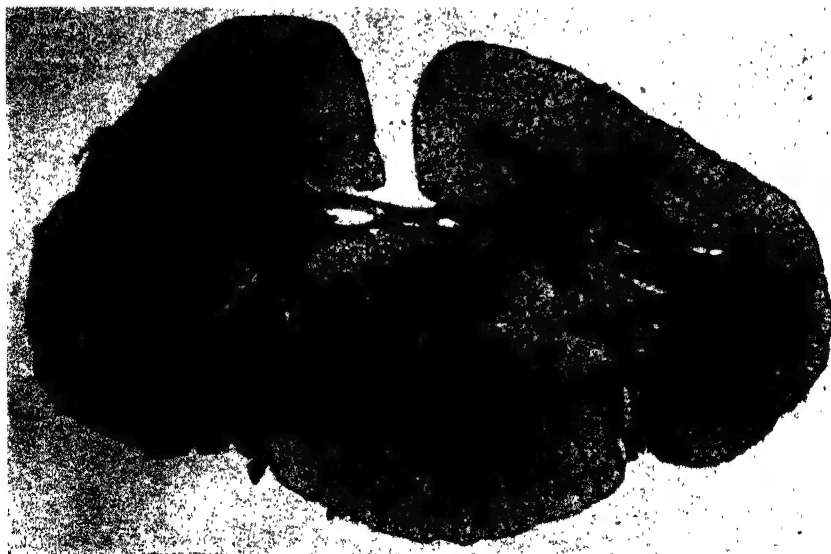


FIG. 203. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig. 202. Holzer stain.

Pakenham-Walsh in a personal communication states that of three cases treated with wheat germ one has definitely improved and the others did so for a short time, and then continued to degenerate. Gutiérrez-



FIG. 204. Case I. Slight insular gliosis from a treated case of amyotrophic lateral sclerosis. Compare with Fig. 205 from an untreated case. In Figs. 205 the gliosis is dense; Holzer stain. $\times 200$.

Mahoney [151] cured six of nine cases. Three relapsed when they ceased to take wheat germ oil concentrate, but again recovered when they resumed. One man, who could only walk with a stick, after two years' treatment ran well and returned to work.

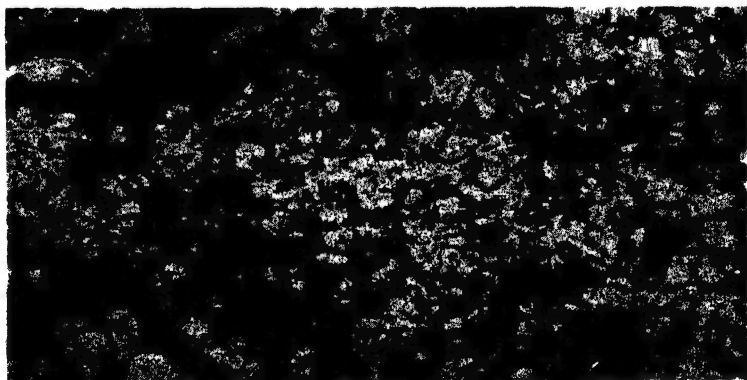


FIG. 205. From an untreated case of amyotrophic lateral sclerosis, for comparison with Fig. 204. Holzer stain. $\times 200$.

Weinberg [146] gave wheat germ oil to one man who had developed signs suggestive of amyotrophic lateral sclerosis while taking sulphathiazole. The neurological condition was cured though the concentration of the sulphathiazole in the blood remained unchanged. Other vitamins failed to prevent the recurrence of neurological signs each time the drug was given. This is reminiscent of the effect of sulphathiazole in accelera-

ting the development of muscular dystrophy in vitamin E deficient animals (p. 716), though not in man (p. 768).

Davison [282], who has very kindly allowed us to reproduce his most important and unique photomicrographs in Figs. 192 to 205, treated ten cases—six men and four women—in the manner advised by Wechsler. After death histopathological examinations were made, forty untreated cases being used as controls. In the six cases who were considered to have had adequate treatment for a sufficient period of time—five weeks to over seven months—the degeneration of the cells of the bulbar nuclei and anterior horns was not affected. But the other pathological changes were checked or reversed. The demyelination of the crossed pyramidal tracts was not one-tenth of that in the untreated cases and, indeed, was hardly visible to the naked eye in stained preparations. The changes in the axis cylinders were also far less pronounced. The dense gliosis, plainly visible in stained preparations from the untreated cases, was virtually absent and in most cases the fatty deposits were also greatly reduced. This outstanding work suggests, like that on the muscular dystrophies, that while vitamin E may play an essential part in treatment, it is not in most cases the whole answer to the problem.

Worster-Drought and Shafar [147] have reported that synthetic vitamin E in 80 mg. doses daily had no effect in twelve cases, apart from one who appeared slightly better. They noted that mental depression was relieved. Fitzgerald and McArdle [182] treated ten cases with no good results except occasional subjective improvement while in hospital. Apparently treatment consisted of synthetic vitamin E, and synthetic vitamin B₆ in varying doses, given by mouth or by injection, and also wheat germ oil. Details are left vague, and presumably the patients on leaving hospital returned to a diet poor in everything except the vitamin E they were given. It seems that it was after leaving hospital that they became worse.

Doyle and Merritt [184] gave eight patients an excellent diet, the vitamin B complex, cod liver oil and 7 c.c. of wheat germ oil daily all by mouth, and synthetic vitamins E (60 mg. daily), B₁ and B₆ and liver extract by injection. The results were negative in both the late and early cases. Sheldon and his colleagues [185] also failed to improve ten cases treated with 45 c.c. of wheat germ oil with each meal, supplemented daily by 50 mg. of synthetic vitamin E by mouth or 100 mg. twice weekly by injection. A later report following more intensive therapy on further cases is equally negative [179].

Denker and Scheiman [148] have reported that eleven patients, including one vegetarian, did not respond to about 100 mg. daily of synthetic vitamin E given by mouth or by injection. Four further patients given 250 mg. daily and 100 mg. of vitamin B₆ did not improve. There was no evidence, either from the histories, gastric analyses, or radiography, that intestinal disease was hindering absorption. Ferrebee, Klingman and Franz [186] gave wheat germ and synthetic vitamins E and B₆ by mouth and also synthetic vitamin E by injection to six patients with no results. About 200 mg. of vitamin E were being taken daily.

Goodhart, of the Montefiore Hospital of New York, tells us that twelve

cases treated with synthetic vitamins E and B₁ showed no improvement. De Jong [178] has only noted at best a fleeting subjective improvement in twenty-six patients treated for upwards of a year with wheat germ oil, yeast, and injections of alpha-tocopherol up to 240 mg. daily. In the discussion following De Jong's paper Moersch and Viets reported, without giving details of treatment, another thirty-eight cases among whom only one improved. Furtado and De Carvalho [249] also failed to benefit twelve patients treated with wheat germ oil or alpha-tocopherol for one to twenty-seven months.

Tabes Dorsalis. Bicknell [9] reported two cases of advanced tabes dorsalis with severe lightning pains who were not improved by wheat germ, but Vøgt-Møller [7] believes vitamin E (? whole wheat germ) is of some value combined with other treatment. Stone [194], in a paper which should be read in full, reports the excellent results he obtained by treating eighteen cases with wheat germ oil and the vitamin B complex. All had previously had routine treatment without arresting the progress of the disease. Improvement chiefly occurred in the gait, power, co-ordination, bladder function, and frequency of gastric crises and lightning pains.

Disseminated Sclerosis and Other Conditions. Bicknell reports that one case of familial periodic paralysis with severe cord changes has slightly improved and one case of myotonia congenita is considerably better, now going to work when three years ago he was confined to a chair. A second case has not responded to treatment. Meller [174] found that large doses of alpha-tocopherol are valueless in disseminated sclerosis. Couperus [255] in Holland has reported negative results from the treatment of all the conditions mentioned above. Vøgt-Møller [7] states that vitamin E (? wheat germ) is of value in mild cases of myelopathy associated with pernicious anæmia, but Spies and Vilter [140] found an injection of 500 mg. of alpha-tocopherol had no effect on a patient with subacute combined degeneration, though this treatment caused a slight reticulocyte response in three patients with macrocytic anæmia. Spies and his associates [142] thought that synthetic vitamin E improved a severe case of arsenical peripheral neuritis. The use of the vitamin has been suggested in the prevention of infantile cerebral palsies [151], and infantile muscular atrophy of spinal origin [180]. Vitamin E is said to be of value in post-diphtheritic paralysis [181] and otosclerosis [182].

Congenital non-obstructive hydrocephalus has been treated by Stone [245] with wheat germ oil. In all his nine infants the progress of the hydrocephalus was checked and there was marked improvement in the mental condition, in the visual acuity and in the consistency of the leg muscles. In two infants successful operations were performed on meningo-coeles and in a third a broken-down meningomyelocoele healed. The use of vitamin E for reducing capillary permeability has considerable experimental justification from the work of Dam (p. 784).

Sabin and Duffy [105] from experimental work on young mice believe that vitamin E plays a part in the resistance of the nervous system to virus infections and Vøgt-Møller (p. 786) comes to the same conclusion from a study of distemper in dogs. Einarson and Ringsted's [6] experimental work stresses that the lower motor neurons degenerate when there is a

deficiency of vitamin E. These observations suggest that vitamin E might be of value in increasing the resistance of children to infantile paralysis.

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CHAPTER IX

ESSENTIAL UNSATURATED FATTY ACIDS

(VITAMIN F)

AND OTHER MINOR FAT SOLUBLE VITAMINS

LINOLEIC, linolenic and arachidonic acids—the essential unsaturated fatty acids—are still occasionally referred to by their American name of vitamin F, though there is general agreement that this name is unsuitable [1] and liable to cause confusion, since it was used at one time for part of the vitamin B complex, while factor F₁ and F₂ are still used for substances found in the urine which are related to nicotinic acid.

Even the name “essential unsaturated fatty acids” is ambiguous, since any one of the three acids which it includes can replace both the other two in the diet.

History. Burr and Burr [2] in America in 1929 and 1930 first described the “fat deficiency disease” of rats which occurs when the diet is deficient in certain polyunsaturated fatty acids, though for some years before this it was becoming increasingly plain that the body cannot satisfy all its fat requirements by synthesis from the carbohydrates of the food. Further work in America and in England—especially by Smedley-Maclean and her colleagues—has confirmed and amplified the experimental work of Burr and Burr and has shown that other species beside the rat require these poly-unsaturated fatty acids. For man, however, their importance is still uncertain, though their deficiency is probably one of the causes of infantile eczema.

Chemistry. The three essential unsaturated fatty acids are :—

Linoleic acid—



Linolenic acid—



and Arachidonic acid—



The first two of these are colourless oils while arachidonic acid forms colourless crystals with a melting-point of 77° C. All three are soluble in organic solvents and alkalis but not in water. Their particular chemical properties depend on the positions of their double bonds. For details of their isolation from fats and their chemical reactions and estimation the reader should consult the books by Smedley-Maclean [3], Hilditch [4] or Rosenberg [5].

From the dietetic point of view the most salient property of the unsaturated fatty acids is the ease with which they become rancid, when exposed to the air, through oxidation at their double bonds. The first

products of this oxidation are labile peroxides which alter further into keto-hydroxylic derivatives and then either polymerize or undergo disruption with the formation of aldehydic compounds [4]. These products of oxidative rancidity are of the greatest importance since they are not only toxic in themselves [6], causing for instance anæmia [7], but also their presence in food leads to the oxidation and destruction of other substances, so that they may convert diets containing ample carotene and vitamin A (p. 42) or ample vitamin E (p. 729) or ample of the vitamin B complex [8] into diets deficient in these vitamins.

Physiology. Synthesis. Linoleic and linolenic acids are found in large quantities in vegetable fats, but the presence of arachidonic acid is uncertain [4]. In fish oils [4, 9, 10, 11] it is doubtful whether any of the three essential unsaturated fatty acids occur in forms identical with those found in vegetable and animal fats unless, which is very rare, the fish have consumed large amounts in their diet [4]. None of the warm-blooded animals so far investigated can synthesize linoleic or linolenic acid, though either of these can be converted in the body into arachidonic acid or clupanodonic acid [12]—a higher and more unsaturated fatty acid.

Absorption. No particular work has been done on the absorption of the essential unsaturated fatty acid, but there is no reason to suppose it differs from that of other fatty acids.

Storage, Utilization and Excretion. Linolenic acid is not found in animal tissues [18] unless very large amounts are being consumed [8], but in normally fed animals linoleic and arachidonic acids are stored in large quantities, especially in the phospholipoids. Linoleic rather than arachidonic acid tends to be stored in the depot fat; in man, for instance, linoleic acid forms 8.2 to 11.0 per cent. of the total fatty acids, while arachidonic acid forms only 0.8 to 1.0 per cent. [14]. In lecithin from bovine liver [15] linoleic acid forms 45 per cent. of the unsaturated fatty acids and arachidonic acid 81 per cent.; the latter acid forms 5.5 per cent. of the total fatty acids of the suprarenals and 4 per cent. of those of the spleen, while for the liver and thyroid of the pig the figures are 2.0 to 7.7 per cent. and 0.4 per cent. [16]. The skin and subcutaneous tissues of the newly-weaned rat contain 0.88 per cent. of poly-unsaturated acids and the carcass fat 1.55 per cent. [17], while the livers of adult rats contain 8 to 7 per cent. [4]. When arachidonic acid is added to a deficient diet the stores in the liver are replenished before those in the body fat [17].

The essential fatty acids are most carefully husbanded in the body. Thus Smedley-Maclean and Hume [17], for instance, report that after nearly six months on a deficient diet, when all growth has ceased (p. 787), the skin and subcutaneous tissue contains traces of the polyunsaturated acids while the total content of the carcass fat is still more than half of what it was at weaning. Even after a further six months on the deficient diet there is little further depletion and the liver contains about 0.8 per cent. of these acids, probably as dihydroarachidonic acid [12].

Little is known about what activities of the body lead to depletion of its stores of the essential unsaturated fatty acids, though probably growth is the most important. Thus when deficient rats are given arachidonic

acid, so that growth is resumed, a large proportion of the acid cannot be recovered from the animals [10]. Rapidly growing tumours decrease storage—though they grow normally even when storage is very low [18]. During lactation there is some loss through secretion in the milk. The various physiological and pathological conditions which have been stated to alter the amount of unsaturated fatty acids in the serum have been reviewed by Hansen [46].

Activity of Fatty Acids. "Fat-deficiency disease" is an unfortunate name since the disease is not due to a deficiency of fat in general, but to a deficiency of the essential unsaturated fatty acids. The disease can neither be prevented nor cured by saturated fatty acids [19, 28], by oleic acid [21], by isomeric oleic acid [21] with the double bond at 12:18, by alpha-elæostearic acid [22], by erucic acid [21], by chaulmoogric acid [21, 28], by ricinoleic acid [21, 28], or by some of the oxidation products of linoleic and linolenic acids [28].

Cod liver oil [10], salmon egg, pilchard and herring oil [11] have little or no effect, though one of the highly unsaturated acids of cod liver oil promotes growth [28].

Prevention and cure of the fat-deficiency disease is brought about by linoleic acid, linolenic acid, some of the oxidation products of these [28], and arachidonic acid. The activity of the first two of these appears to depend on having double bonds at 9:10 and 12:13, but not on their carboxyl group, since this may be changed for an alcohol group [21]. Arachidonic acid is about three times as potent as linoleic acid for promoting growth [21] and linoleic acid is about six times as potent as linolenic acid [28]. For normal gestation linolenic acid is less effective than the other two acids [89]. For curing the skin symptoms of the fat deficiency disease, however, linoleic and arachidonic acids are equally efficacious [10], and this, combined with the fact that docosahexænoic acid from cod liver oil promotes growth but has little or no effect on the skin [28], has led Hume and others [28] to the conclusion that the essential fatty acids have not identical actions.

The daily requirements of young rats, judged by their growth, is about 100 mg. of linoleic acid or 38 mg. of arachidonic acid [21]. Other workers [81] have reported smaller requirements, probably because they have adopted less stringent criteria for the adequacy of their diets.

Symptoms of the Fat Deficiency Disease. Weanling rats have been used almost exclusively for studying the effects of a deficiency of the essential unsaturated fatty acids, though isolated reports suggest that the mouse [24] and the dog [25] respond to a deficiency in the same way as the rat. Observations on man are described on p. 790.

The symptoms and signs of the fat deficiency disease are retarded and ultimately arrested growth—accompanied by a raised metabolic rate—altered fat and water metabolism, changes in the skin and hair, renal degeneration and impairment of the sexual functions.

Growth. During the first three or four months of the deficient diet growth continues though at a diminished rate; after this the weight remains stationary or declines slightly though the animals may survive for many months [2, 17, 21, 28]. The consumption of food, however, remains

as high as that of animals on a normal diet [2], so that the basal metabolic rate is considerably increased [26].

Fat and Water Metabolism. Burr and Burr [2] originally believed that deficient animals could not synthesize fat, but it is now known that fat metabolism remains virtually normal. Fat is synthesized from carbohydrate since after a carbohydrate meal the respiratory quotient rises above unity [26] and this strong evidence for the synthesis of fat is confirmed by Smedley-Maclean and others [17] finding that the proportion of fat in the subcutaneous tissues and, to a lesser extent, in the carcass is actually higher in deficient than normal animals while in the liver it remains normal [17] or raised [12]. That fat is burnt normally as shown by the low respiratory quotient of fasting deficient rats [85]. The rate of synthesis [27, 28] and breakdown [27] of phospholipoids remains normal in the liver [27, 28] and kidney [27] of the deficient animal, while it is increased by about one-third in the muscles [27].

The sparing action of fat on vitamin B₁ (p. 167) is not affected by the degree of unsaturation of the fat [29].

The lipotropic effect of choline depends, according to Engel [80], on the presence of linoleic acid. But this is probably a secondary effect due to the acid permitting growth and so fat consumption [18].

About twice the normal amount of water is drunk by the deficient animals although there is no increase in the amount of urine [2].

Skin and Hair. Both Burr and Burr [2] and Hume and her collaborators [28] report that dryness and scurfiness of the fore and hind paws is the earliest and most constant symptom of the deficiency, occurring while the animals are still growing, after only ten days to a few weeks on the deficient diet. The condition of the paws returns to normal within three to five weeks when linoleic or arachidonic acid are given (p. 787). The skin over the rest of the body also becomes dry and scurfy and the hair thin, especially over the face and round the eyes [21]. The tail may be hairless and corrugated or annulated or even necrotic, but these changes are inconstant and irregular and heal very slowly [28]. Some observers [21] even find all skin changes are slight and unreliable indicators of the deficiency—a finding possibly due to the climate of different laboratories, since the type of weather which causes chapping in man accentuates the skin changes in the deficient rats [26, 81]. Vitamin B₆ has been thought to have some relationship to the essential unsaturated fatty acids, since its deficiency also causes somewhat similar changes in the skin of rats, but the only relationship is the obvious one that a deficiency of both will cause grosser skin changes than a deficiency of only one [82]. The same applies to pantothenic acid. Vitamin B₆ does not enable the animal to synthesize linoleic or linolenic acids [88].

Williamson [84] reports that the epidermis of the fat deficient rat becomes thicker and more differentiated, the *stratum granulosum* being especially distinct and the horny layer thick. Cells showing mitosis may be four or even eight times as numerous in rats kept on the deficient diet for nine weeks as in normal rats [8].

Renal Degeneration. Hæmaturia was a frequent finding in the rats originally described by Burr and Burr [2], indeed these workers have since

reported renal lesions in all their animals [81]. Other workers, however, have frequently failed to find any hæmaturia in rats [21, 28], and no renal lesions in dogs [25]. The lesion in the kidney is said to be calcification in the cells of some renal tubules and necrotic areas in the renal medulla [86]. Burr and Burr [2] consider the renal lesion, which is made worse by a high protein diet, is the cause of death.

Sexual Functions. Ovulation, though rarely it may remain normal [21], is generally grossly disturbed or completely absent [2, 21]. When it is absent it will start again within five days of giving the essential unsaturated fatty acids [2]—this response is as rapid as that of spayed rats to oestrone. Mating may take place if ovulation occurs, but gestation is prolonged and ends in resorption in one-fifth of the animals [87], or in protracted labour with excessive hæmorrhage [89]. The litters are undersized and often so weak that they soon die [8, 87]. Lactation is possible if the mothers are given the missing fatty acids, but is poor unless other fat is also added in reasonable amounts to the diet [87].

Males show a loss of the normal sexual responses and will seldom mate; when they do they are sterile [2]. This sterility can be cured [88]. After nine months on the deficient diet most of the tubules of the testis are lined with spermatogonia and one or more layers of spermatocytes, no maturer cells being present. Considerable numbers of tubules have no epithelium and multinucleated giant cells may be found in the lumen [88].

The relation between the unsaturated fatty acids and vitamin E in the nutrition of the chick is discussed on p. 734.

Distribution in Foods and the Effects of Storage and Hydrogenation. Vegetable and seed fats, though not margarine, may contain large amounts of linoleic and linolenic acid, but for all practical purposes contain no arachidonic acid. The highly unsaturated acids of fish oils appear not to contain the essential fatty acids in the forms found in land plants and animals, cod liver oil and other fish oils having little effect on the cure of the fat deficiency disease (p. 787), though one of the highly unsaturated acids of cod liver oil does bring about a resumption of growth (p. 787). Animal fats, according to the diets of the animals, may contain large amounts of the essential unsaturated fatty acids (p. 786), and human milk [67], in contrast to cow's milk, is an excellent source, providing as well higher unsaturated fatty acids.

The ease with which the essential unsaturated fatty acids are destroyed by oxidative rancidity in the presence of air (p. 785) means not only that they themselves may be destroyed when food is not fresh, but also that their rancidity may destroy other essential constituents of the diet (p. 786) besides the vitamins (p. 786). The hydrogenation of the vegetable fats of margarine, while it has the advantage of enabling manufacturers to sell a product which delights shopkeepers by remaining tasteless for many months in a warm room, has, of course, the drawback that much of the essential unsaturated fatty acid is converted to saturated fatty acid.

The following figures have been largely taken from *The Chemical Constitution of the Natural Fats* by Hilditch [4] to which the reader should refer for further information.

THE VITAMINS IN MEDICINE

Food.	Per Cent of Essential Unsaturated Fatty Acid.
<i>Animal Products (p. 786) ;</i>	
Butter	1·9-4·0
Beef Fat	1·1-5·0
Lard	5·0-11·1
Mutton Fat	8·0-5·0
Liver Fat	8·0-7·0
Milk (Cow)	0·15-0·23
(Ewe)	0·36
(Goat)	0·22
(Human)	0·89-0·40
(Mare)	0·69
Fish Oils	Traces (p. 787)
Margarine.	2·0-5·0
<i>Vegetable Fats :</i>	
Barley Germ Oil	63
Cocoa Butter.	2·0
Coconut Oil	6·0-9·2
Corn Salad Oil	70
Cotton Seed Oil	85-50
Ground Nut or Arachis Oil	18-27
Linseed Oil.	72-83
Maize Germ Oil	42
Oat Germ Oil	81
Olive Oil	4·0-13·7
Palm Oil	2·0-11·3
Rice Bran Oil	29-42
Rye Germ Oil	48
Soya Bean Oil	56-63
Sunflower Seed Oil	52-64
Wheat Germ Oil	44-52

Human Requirements of the Essential Unsaturated Fatty Acids.

Nothing is known about the human requirements of these acids. One human volunteer [48] has lived for six months on an almost fat-free diet, which produced the fat deficiency disease in rats, with no ill results, though, as might have been expected, he lost weight, his respiratory quotient altered and there was a marked fall in the level of linoleic and arachidonic acid in the blood. The frequent attacks of migraine from which he suffered were permanently cured. Of seven infants reported in the literature [6] who were given diets very low in fat, two developed eczema which was then cured by fat and there is some clinical evidence, discussed below, that eczema, especially in infants, is associated with abnormally low levels of unsaturated acids in the blood and may be improved by adding unsaturated fatty acids to the diet. The condition of the skin and especially of the hair in coeliac disease and other diseases where fat absorption is impaired, in which the level of the unsaturated fatty acids of the serum is low [44, 46], may also possibly point to the unsaturated fatty acids being necessary in human nutrition.

But when it is remembered that rats show few definite symptoms of the fat deficiency disease until they have been several months on the

deficient diet and when it is also remembered how high are the stores of the essential unsaturated fatty acids in man (p. 786), it is hardly surprising that neither deliberately planned deficient diets nor diets low in fat such as those produced by poverty, or during war in England, cause any definite symptoms clearly ascribable to lack of the essential fatty acids.

While, however, there appears to be no frank deficiency of these acids in human nutrition, it is relevant to remember that in processed and stale food they may be deliberately destroyed to improve the keeping quality of the food or accidentally destroyed by rancidity, while changes from the traditional feeding of cattle and poultry may alter the amounts in animal fats and dairy produce [4].

Essential Unsaturated Fatty Acids in Medicine

Eczema. The only condition where these acids appear to have some definite value is eczema. Hansen [45] found that the average iodine number of the serum fatty acids of eleven infants with eczema was 82, while in eleven normal controls it was 114. When the eczematous infants were given linseed oil or corn oil they all improved. The dose of the oil and details of the rate and degree of improvement are not given, though apparently three weeks or even much longer is required for the skin and iodine number of the blood to return to normal. In four infants, who were intensively studied and well controlled in hospital, clinical improvement coincided with the rise in the iodine number of the serum. In a later paper Hansen [46] has given an account of his own extensive work on the serum lipoids of one hundred and forty patients, half of whom were normal while the other half suffered from various diseases including eczema. He has also reviewed the literature on the many causes of alterations in the iodine number of the serum lipoids. Cornbleet [47] gave eighty-seven patients with allergic eczema, most of whom were adolescents or young adults in whom the condition had been present for many years, four tablespoonfuls three times a day of maize oil. Cure took twelve to eighteen months, and followed an erratic course; concomitant asthma also often improved. Faber and Roberts [48] confirmed the low iodine number in the serum lipoids of infants with eczema, but could not confirm the curative properties of unsaturated oils. Taub and Zakon [49] treated eczematous patients with raw linseed oil and reported that some were worse after the treatment, while Ginsberg and others [50] failed to improve the eczema of infants and of adults with corn oil and linseed oil, and found no difference in the iodine number of their serum lipoids compared to that of normal subjects. The work of Finnerud and his colleagues [51] may explain these contradictory results since only half of forty-seven eczematous patients had low iodine numbers and it appears that it was especially in all these patients that definite improvement occurred after taking three tablespoonfuls of lard daily, which apparently were administered by being used in the cooking. In the patients with normal serum values improvement was less constant. The level of arachidonic acid in the blood was never low, even when the iodine number was low.

Burns and Wounds. Cod liver oil is the oil which has been most extensively used in the treatment of burns and wounds, but though it contains a large proportion of highly unsaturated fatty acids, these are not, to any

marked degree, the "essential" unsaturated fatty acids (p. 787). Löhr and Zacher [52] first drew attention to the use of cod liver oil ointments for treating burns owing to the excellent results which they obtained in a large number of cases; they especially stressed the rapid cleaning and epithelization. Steel [58] confirmed these results both for burns and wounds, being particularly impressed by the stimulation of indolent areas. Dann and her collaborators [55] have carried out the most thorough experimental work yet published, and have given an extensive bibliography to German clinical work. They report that cod liver oil, arachis oil and linoleic acid all stimulate granulation tissue, though only the last stimulates epithelial regeneration. Arachis oil causes excessive collagen formation. Puestow and his colleagues [54], from excellent experimental work on pigs and rabbits, concluded that burns treated with fish liver oils healed twenty-five per cent. more rapidly than burns which received no treatment or olive oil. The effect was not due to the vitamins in the fish liver oils, and it is interesting to note that the "essential" linoleic acid of the olive oil had no effect. Contradicting these reports is the slighter clinical and experimental work of McClure and Lam [55] who found cod liver oil of no value in the treatment of burns in men and guinea-pigs.

Tuberculosis. Here again it is cod liver oil and not the unsaturated vegetable oils which has won for itself a wide reputation in treatment. It is difficult to understand why cod liver oil has this reputation, it is more difficult to ignore it. For over half a century physicians have been impressed by it both in England [56] and in America [57], and though at the present time it receives scant attention in medical journals, yet when it is mentioned it is praised, McConkey [57], for instance, reporting after well-controlled work that it gives remarkably good results in preventing laryngeal and intestinal tuberculosis in cases of pulmonary tuberculosis.

OTHER MINOR FAT-SOLUBLE VITAMINS

Butter Growth Factor. The review by Burr and Barnes [6] in 1948 gives an excellent brief summary of, and references to, the earlier conflicting evidence that butter fat contains a factor which promotes growth. There can be little doubt that butter fat for the calf is superior to all the common vegetable oils and, to a lesser degree, to animal depot fats [58]. For the rat, which is the animal that has been most extensively studied, Boutwell and his collaborators [59] in their first papers reported that butter is superior to maize, coconut, cotton seed or soya bean oils, this superiority apparently being due to the presence of long chain saturated fatty acids. But in later papers these workers stated that the superiority of butter vanishes when mixed carbohydrates instead of lactose alone were given in the diet [60, 61]. On the lactose diets the flavour of the butter was not the factor which was responsible for the better growth and food consumption, because removing the diacetyl—the substance which largely gives butter its distinctive flavour—from the butter did not decrease the effect of the butter nor was the relative effect of corn oil reversed by flavouring it with diacetyl [61].

Deuel and his collaborators [62] have confirmed that young rats prefer

and consume far larger quantities of diets containing butter than diets containing vegetable oils. They have also shown that vegetable oils flavoured with diacetyl are preferred to oils without this flavour, but they have not confirmed or denied the crucial work of Boutwell and others [61] that even when vegetable oils are flavoured they are inferior to butter. Their experiments [68] in which they showed that rats consuming *the same amounts* of diets containing butter or other fats grew equally well and had the same carcass composition, are of little value since these by ensuring that the same amount of food was eaten by all the rats, ignored the essential fact that stimulation of appetite is one of the most important properties of food and of vitamins such as B₁.

On the balance of evidence it would appear that butter, at least for calves and young rats, is superior to vegetable fats for promoting appetite and growth when lactose, the normal carbohydrate for young animals, is the only carbohydrate in the diet.

Anti-Stiffness Factor. Bahrs and Wulzen [64] in 1936 noticed that planarian worms became diseased when fed on the tissues of guinea-pigs whose vitamin C requirements had been supplied by tomato juice, orange juice or synthetic ascorbic acid. When, however, fresh green kale was used to provide vitamin C the tissues of the guinea-pigs caused no disease in the worms.

Following up this indication that kale contained a new vitamin, Wulzen and her collaborators [65, 66] showed that guinea-pigs develop a definite deficiency disease when fed on a diet of grain and the "necessary" vitamins, or on a diet of skim milk supplemented with straw, orange juice, carotene and a salt mixture.

The dominant symptom of the disease is stiffness of the wrists which gradually grows worse and also in time involves the elbows. At autopsy the muscles are extremely atrophied and are, in most cases, streaked with closely-packed fine white lines of calcium deposits running parallel to the muscle fibres. There are often lumps of calcium phosphate deposited under the skin, in the region of the joints, between the ribs and indiscriminately in many organs including the heart and aorta. This deposition of calcium phosphate appears to be due to the increase in the blood of both calcium and inorganic phosphorus [68]. The serum phosphatase is decreased [68] and there is an abnormal distribution of the acid soluble phosphorus in the kidney and liver [69]. This disease is quite different to the muscular dystrophy caused by lack of vitamin E, since in animals suffering from both diseases either one can be cured separately and, further, the "stiffness" disease, which does not cause creatinuria, is cured without reducing the creatinuria of the muscular dystrophy [66].

Cod liver oil accelerates the onset of the symptoms and aggravates the condition. Neither the grass juice factor of Kohler, Elvehjem and Hart, nor vitamin E, in the form of wheat germ oil or synthetic alpha-tocopherol, cures or prevents the condition [66]. Methyl vinyl ketone, in spite of a report to the contrary [64], also has no curative effect [65].

On the other hand fresh kale or alfalfa prevents and cures the disease, and so does fresh raw cream, of which 1 gram daily for five days alleviates the stiffness of the wrists. Heating the cream or pasteurizing it in the

presence of oxygen, but not of nitrogen, destroys its curative power. From raw cream the anti-stiffness factor has been extracted, in a nearly pure form, which in daily doses of 0.1 micrograms cures the stiffness of the wrists in five days. The factor has now been isolated in a crystalline form. It is fat soluble, has a molecular weight of about 200, and contains one carbonyl group [66].

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CHAPTER X

VITAMIN K

PHYLLOQUINONE

HISTORY

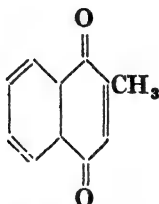
BETWEEN 1929 and 1933 several observers described a hæmorrhagic disease in chicks fed on diets poor in fats. The existence of a vitamin deficiency as a cause of the hæmorrhage was suspected in 1929 by Dam [1] of Copenhagen, and later in 1931 by McFarlane and his collaborators [2, 3] in their work on the fat-soluble vitamin requirements of the chick. At first it was thought that the hæmorrhagic syndrome was related to scurvy, although it is known that the chick synthesizes its own vitamin C. Cabbage, which contains this vitamin, was found to cure the hæmorrhagic condition [4], but supplements of lemon juice, pure ascorbic acid, cod-liver oil and wheat-germ oil failed to do so, showing that the condition was not due to deficiency of vitamin C or to any of the then known fat-soluble vitamins [5]. In 1934 Dam [5] suggested that this hæmorrhagic tendency in chicks was a definite dietary deficiency disease due to lack of a fat-soluble factor, and in the next year he proposed that this factor be called vitamin K (Koagulations-Vitamin), a name that has been generally adopted.

The first definite proof of the fat-soluble nature of vitamin K was supplied in 1931 by McFarlane and his co-workers, who noted that fish meal cured the hæmorrhagic disease in chicks on basal diets, but did not do so if the meal was first extracted with fat solvents. The fat-soluble nature of the vitamin was conclusively proved by Dam [5], Almquist and Stokstad [6], who obtained crude concentrates of the vitamin by the extraction of certain foodstuffs with ether.

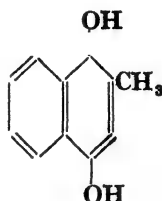
The relationship between the new vitamin and the clotting of blood was established when McFarlane [8] and Schönheyder [7] observed that the blood of chicks suffering from the nutritional hæmorrhagic syndrome had a prolonged clotting time. In 1936 Schönheyder [8] first suggested that the condition resulted from hypoprothrombinæmia. This was proved by Dam, Schönheyder and Tage-Hansen [9], who were unable to isolate prothrombin from the blood of vitamin K deficient chicks, although it was found to be present in the blood of normal chicks. They also noted that an aqueous emulsion of a vitamin K concentrate failed to restore clotting time to normal when added to a blood plasma-thromboplastin mixture, thus showing that the prolonged clotting time was due to a low plasma prothrombin level in the vitamin K deficient chicks, and that vitamin K itself had no thrombin-like activity. Quick [10] in 1937 observed a progressive fall in the prothrombin level in the blood of chicks on a vitamin K deficient diet. A distinct hæmorrhagic tendency appeared when low prothrombin levels were reached, and both the low prothrombin and the hæmorrhagic tendency were cured by the administration of foodstuffs rich in vitamin K.

Finally in 1937 it was shown that mammals and man may develop

Unlike vitamins K_1 and K_2 it is water soluble, and forms yellow prismatic crystals melting at 178°C . Most of the analogues are derivatives of 1:4-naphthoquinone or 1:4-naphthohydroquinone. A synthetic naphthoquinone that has been exhaustively studied is 2-methyl-1:4-naphthoquinone, which is official in the B.P.C. as menaphthone. In the U.S.A. it is known as menadione.



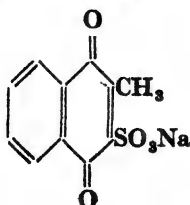
2-Methyl-1:4-naphthoquinone
(Menaphthone, Menadione.)



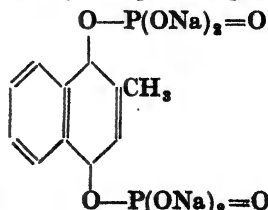
2-Methyl-1:4-naphthohydroquinone

This has a high antihaemorrhagic potency, and weight for weight it is more potent than vitamin K_1 itself, although on a molar basis the natural vitamin is the more potent. Allen [176] states that clinically it is more potent than natural or synthetic vitamin K_1 . Figures on the potency of various compounds are liable to be misleading as they are expressed in units per gram of substance. To obtain really comparable results antihaemorrhagic activity should be expressed in units per gram molecule. Many of the analogues of vitamin K_1 have a much lower molecular weight, and therefore when comparisons are made on a simple weight basis they appear more potent. 2-Methyl-1:4-naphthoquinone is only slightly soluble in water. Its activity is impaired by exposure to light or by sterilization with steam for any length of time. Apparently it is absorbed when applied cutaneously in a fatty base [189]. It is not suitable for intravenous use because of its oily character.

A number of derivatives of 2-methyl-1:4-naphthoquinone have been prepared. 2-Methyl-1:4-naphthohydroquinone diacetate (acetomenaphthone, B.P.C.) is more stable than 2-methyl-1:4-naphthoquinone, but has the disadvantage of not being water-soluble. 2-Methyl-1:4-disuccinyl-naphthohydroquinone is soluble, but it is readily hydrolysed and the solution must therefore be freshly prepared before use. This objection has been overcome by preparing the sodium salt of 2-methyl-1:4-naphthohydroquinone diphosphoric acid ester [28], or the sodium salt of 2-methyl-1:4-naphthoquinone-3-sulphonic acid [107, 169, 179, 280, 281, 246], which are water-soluble and stable. The diphosphoric ester is stated to be twice as active and less toxic than 2-methyl-1:4-naphthoquinone [187, 188].



Sodium salt of 2-methyl-1:4-naphthoquinone-3-sulphonic acid.



Sodium 2-methyl-1:4-naphthohydroquinone diphosphoric acid ester.

It has been shown in both the experimental animal and in human beings that water-soluble vitamin K analogues are absorbed from the gastro-intestinal tract without the aid of the bile salts usually given with natural vitamin K and its fat-soluble analogues [50, 51]. Smith and Owen [51] have shown that 4-amino-2-methyl-1-naphthol (vitamin K₃), which is water-soluble, is physiologically active without bile salt medication. 4-Amino-2-methyl-1-naphthol and 1-amino-2-methylnaphthol hydrochlorides are stated to be more active physiologically than 2-methyl-1 : 4-naphthoquinone [64, 65]. Other water-soluble analogues that have been used clinically are 1 : 4-dehydroxy-2-methyl-8-naphthaldehyde [148], 2-methyl-1 : 4-naphthohydroquinone bisulphite [174] and 2-methyl-1 : 4-naphthohydroquinone dibutyrate [14].

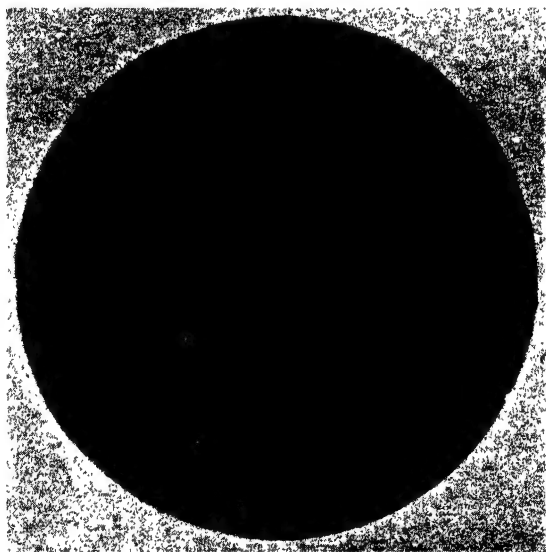


FIG. 206. Crystals of Menaphthone (2-Methyl-1 : 4-naphthoquinone).

An intact benzene ring in the vitamin K molecule is essential for activity ; if it is substituted activity is lost. The 2-methyl group is also essential and cannot be replaced by hydrogen or by other groups without serious loss of activity. The quinone structure is not essential, *e.g.*, the hydroquinones are almost as active, and the quinone oxygen can be replaced by other groups such as amino- and aldehyde. The side chain in the 8-position can be eliminated without loss of activity. In fact the most active compounds are derivatives of 2-methyl-1 : 4-naphthoquinone, in which the side chain is entirely replaced by hydrogen. It has been suggested that vitamins K₁ and K₂ owe their activity to their degradation to 2-methyl-1 : 4-naphthoquinone within the body.

Natural vitamin K and its synthetic analogues have been estimated by: (a) reduction and titration with 2 : 6 dichlorophenolindophenol, using phenosafranine as an indicator, or with ceric sulphate, with *o*-

phenanthroline as indicator [288, 289]; (b) direct titration with excess bromine in carbon tetrachloride, the excess bromine being titrated by means of potassium iodide and sodium thiosulphate solution [289]. A colorimetric test, using sodium diethyl dithiocarbamate has also been devised [240].

UNITS OF VITAMIN K [160]

The methods used for the assay of vitamin K and its analogues are based on the prevention or cure of vitamin K deficiency in chicks. They are based upon changes in the clotting time or upon the prevention of hæmorrhage. Schönheyder [29] and later Dam and Glavind [80] measured the degree of deficiency by the amount of thromboplastin (thrombokinase) necessary to give a constant clotting time of three minutes. The clotting capacity of blood is expressed in terms of R, which equals $\frac{K}{K_n}$. K is the

concentration of thromboplastin required to give a three-minute clotting time of the test plasma diluted with an equal volume of Ringer's solution, and K_n is the thromboplastin concentration required to give the same clotting time for equally diluted normal chick plasma. For normal chicks $R = 1$, whilst for avitaminotic chicks it may be 150 or more.

In the older literature several units are met with, although with the introduction of synthetic vitamin K analogues they are now obsolete.

The *Schönheyder Unit* of vitamin K is defined as the smallest daily dose per gram of chick which will correct the clotting defect in three days [29].

The *Dam Unit* [80] of activity refers to a special preparation of dried spinach, to which a value of 500 units per gram has been arbitrarily assigned. Two mg. of this product therefore constitutes 1 unit. When 2 mg. of this standard preparation per gram of body-weight is given daily to a highly K-avitaminotic chick on three successive days normal blood clotting is obtained.

Both the Schönheyder and Dam techniques are laborious. Thayer, Doisy [81] and their associates have devised another curative assay method. Groups of ten or more deficient chicks, fed on the basal diet for approximately two weeks, are fed the test material directly on three successive days, and on the fourth day the clotting time is determined. The *Thayer-Doisy Unit* of vitamin K is defined as that amount which will reduce the clotting time to ten minutes or less in fifty per cent. of a group of deficient birds. Later the technique was modified so that only a single dose of test substance was given and the clotting time determined eighteen hours later.

Ansbacher [82] dissolves the material under test in cod-liver oil which is given directly to the avitaminotic birds and the clotting time determined six hours later. An *Ansbacher Unit* of vitamin K is defined as the minimum amount which, when administered to a vitamin K deficient chick weighing 70 to 100 gm., reduces the blood clotting time to normal within six hours of administration. Certain of the more complex vitamin K preparations require a longer time than six hours for utilization, and an eighteen-hour test period is used instead.

Another curative assay method has been devised by Dann, [88].

Groups of approximately twenty deficient chicks are given varying levels of the test material orally for three successive days, and then the clotting times are determined. At the same time another group of deficient chicks is treated with a standard vitamin K concentrate, obtained from alfalfa, and assigned the arbitrary value of 5,000 vitamin K units per gram. By comparing the results obtained by feeding the test substance and the standard, the number of units of vitamin K in the former is determined.

A preventive method of assay has been developed by Almquist and his co-workers [84, 85]. In the first method described the test substance was added to the basal diet in varying levels, and that level found which prevented the appearance of gross hæmorrhages in four weeks. Subsequently a modified prothrombin time on whole oxalated blood was used as a criterion of the prevention of hæmorrhage. The results were expressed as the level of test substance per kilo of diet which prevents the development of the hæmorrhagic tendency. Almquist and Klose [82] observed that the reciprocal of the prothrombin time was directly proportional to the logarithm of the vitamin K concentrate given per kilo of diet. From this empirical relationship they determined from a very few feeding tests the vitamin K activity of an unknown material in terms of a standard alfalfa preparation.

No adequate experimental data are available from which the various units of vitamin K can be correlated. The following equivalents are only rough approximations.

1 Ansbacher unit	= 20 Dam units.
1 Thayer-Doisy unit	= 30 Dam units.
1 Almquist-Stokstad unit	= 37.5 Dam units.
1 Dann unit	= 25 Dam units.

100–150 Thayer-Doisy units per kilo of diet are protective by the method of Almquist, Mecchi and Klose [84].

1 gram of pure vitamin K_1 is equivalent to 12,000,000 Dam units.

The relationship between Ansbacher and other units is given in the following table:—

1 Ansbacher unit equals	10 (1938) Dam	Units.
	12½ (1940) Dam-Glavind	
	½ (1938) Dann	
	1½ (1939) Dann	
	20 (1936) Schönheyder	
	1 (1938) Thayer	
	2 (1939) Thayer-Doisy	

After the discovery of naphthoquinone derivatives possessing vitamin K activity, Thayer [86] and his associates suggested that 2-methyl-1:4-naphthoquinone, being the simplest and most potent of these, should be adopted as a standard of reference in tests of vitamin K potency, the biological activity of 1 μ g. of this quinone being defined as 1 standard unit of vitamin K. The potency of 1 mg. of vitamin K_1 is 450 standard units. Ewing [161] has also proposed 2-methyl-1:4-naphthohydroquinone diacetate as a standard, because it is less sensitive to light than methyl-naphthoquinone.

Much of the early clinical literature refers to units of vitamin K. Now

that synthetic analogues are exclusively used clinically there is no need to specify dosage in units.

DISTRIBUTION OF VITAMIN K IN FOODS

Green plants are the richest sources of vitamin K, although moderate amounts are found widely distributed in the animal body. Alfalfa (lucerne) and spinach are rich sources of the vitamin. Other plants with a fairly high vitamin K content are cauliflower, cabbage, carrot tops, kale, soya bean, chestnut leaves, pine needles and seaweed. It is also present in tomatoes, hemp seed, bran and orange peel. The green parts of the plant contain more than the fruits, seeds and roots. Most fruits, except tomatoes, are poor sources of vitamin K. Mountain ash berries and honey contain the vitamin, or at any rate an anti-hæmorrhagic principle [15, 286, 370]. De Lor and Means [870] in tests on five hundred patients have shown that extracts of mountain ash (*Sorbus aucuparia*) stimulate the formation of plasma prothrombin in man. They ascribe the effect to parascorbic acid (Δ -hexeno-lactone) in the berries. Vitamin K is also found widely distributed throughout the animal body. Dam [89] and his colleagues examined chicks on a normal diet and found that the vitamin was distributed in relatively large amounts in all tissues; liver and lung contain the least. Apparently increased stores of vitamin K are found in the tissues of animals receiving a diet rich in the vitamin. The best animal sources of vitamin K never contain more than ten per cent. of that present in alfalfa. Egg yolk contains small but variable amounts of the vitamin. According to Dam [40] human urine contains no vitamin K, even after the consumption of diets rich in the vitamin.

Bacteria can synthesize vitamin K. It has been shown that 0.6 to 2.0 gm. of dried bacteria per kilo of basal diet will protect chicks against vitamin K deficiency; some bacteria are as potent in their vitamin K content as alfalfa. Fæces are rich in the vitamin, its production being attributed to bacterial action, although Andrus [158] from experiments on isolated intestinal loops disputes this. Moulds, yeast and fungi contain practically no vitamin K.

The vitamin K content of some materials is given below:—

	Dam Units per 100 grams.
Putrefied fish meal	90,000
Chestnut leaves	80,000
Cabbage leaves	40,000
Spinach leaves	55,000
Cauliflower	40,000
Nettle leaves	40,000
Fæces	30,000
Alfalfa	20,000 to 40,000
Pine needles	20,000
Different kinds of algæ	13,000 to 17,000
Tomato, green	10,000
" ripe	5,000
Liver (pork)	5,000 to 10,000
" (poultry)	300

	Dam Units per 100 grams.
Peas, fresh	3,500
Rose hips	2,800
Strawberries	2,250
Carrots	1,000
Eggs from chickens fed on diet rich in alfalfa	1,000
Potatoes	1,000
Fish meal	<500
Cereals	<500 to 4,000
Parsley	200
Milk, cows'	very little
„ human	0-200

PHYSIOLOGY OF VITAMIN K

Function. Vitamin K is essential for normal blood coagulation. The term "anti-hæmorrhagic vitamin" for vitamin K is misleading. It does not arrest hæmorrhage in normal persons or in hæmophilia, purpura or bleeding diseases. As far as is known vitamin K has only one function in the body. It is essential for the formation of prothrombin, the level of which falls in subjects deficient in vitamin K. Prothrombin, the precursor of thrombin, is a water soluble glycoprotein [886]. Vitamin K is either one of the building stones necessary for the formation of prothrombin, or more likely stimulates the liver to a normal prothrombin production [41]. Actually the evidence is against vitamin K being a component of the prothrombin molecule, since pure prothrombin preparations contain no naphthoquinone structure. According to Quick [270] prothrombin is composed of calcium and two separable components, one of which (A) is stable and appears to be related to the oxidation-reduction systems of the blood, the other (B) being heat labile and inactivated by dicumarol (p. 888). The mechanism whereby vitamin K acts is unknown. It has been suggested that the vitamin takes part in an oxidation-reduction system in which SH-groups are oxidized to -S-S-, which has been postulated to occur in the transformation of fibrinogen to fibrin [20].

The coagulation of the blood results from a series of complex reactions involving the interaction of prothrombin, thromboplastin (thrombokinas), calcium, thrombin and fibrinogen. There is also an opposing mechanism, the inhibition of coagulation, in which plasma antiprothrombin, plasma antithrombin and heparin play a part. Little has been added to our knowledge on the coagulation of the blood since Howell proposed his theory of blood coagulation thirty years ago. It is generally accepted that when blood clots the soluble plasma protein, fibrinogen, is converted into fibrin, the basis of the clot, by the action of thrombin. Thrombin does not occur preformed in the blood, but is only produced when blood is shed by the interaction of prothrombin, calcium, and thromboplastin. Calcium and prothrombin are both normally present in blood plasma; thromboplastin, however, is said to be formed when tissues are injured or when certain elements of the blood, notably the platelets, undergo disintegration. Eagle [271], however, considers that the formation of the platelets in initiating coagulation in shed blood has been exaggerated and that the thromboplastin factor is dissolved in the plasma.

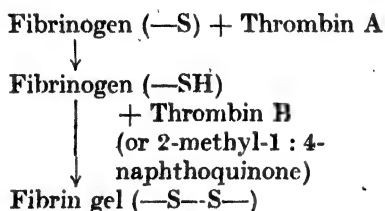
The clotting of blood may be formulated as a two-stage process :—

Prothrombin + calcium + thromboplastin = thrombin.

Thrombin + fibrinogen = fibrin clot.

Lyons [388] has recently advanced a new theory, based on polarographic and chemical analysis of thrombin and fibrinogen, to account for the action of vitamin K in the clotting mechanism. Thrombin can be divided into two active components, A and B, neither of which alone will cause fibrinogen to clot, although a mixture of the two will do so. Thrombin A reacts with fibrinogen liberating thiol groups (SH) to form an intermediat, fibrinogen B, between fibrinogen and fibrin. This has been isolated and can be clotted by thrombin B to form a fibrin gel, or by minute amounts, *e.g.*, 1 μ g., of 2-methyl-1:4-naphthoquinone in 50 per cent. alcohol. In the clotting process it is considered that the thiol groups unite to form disulphide linkages (—S—S—). Microscopical examination of the gels produced by either method reveals no difference between the two. Lyons states that vitamin K is either a prosthetic part of thrombin B, or that it converts a protein fraction into an oxidation-reduction system with a potential in the neighbourhood of that of vitamin K. When digested with trypsin thrombin gives a colour reaction characteristic of naphthoquinone, so that the former suggestion is quite likely.

The reactions postulated by Lyons are illustrated by the following scheme :—



It was formerly thought that calcium in ionized form was indispensable for the clotting of blood, but Quick [270] has recently produced evidence that the calcium is intimately linked with the prothrombin molecule. The reaction between prothrombin, thromboplastin and calcium occurs between pH 6.2 to 8.7 [378]. According to Loomis and Seegers [373] calcium acts as a catalyst and can be replaced by related metals such as strontium.

Heparin also plays a part in the mechanism of blood clotting. It delays coagulation when injected into a normal person, but it is not a natural antagonist to vitamin K, since it affects another part of the clotting mechanism.

In mammals prothrombin is present in large excess over minimal needs for efficient clotting. Thus in dogs the prothrombin may be reduced to one-fifth of the normal level without prolonging the clotting time or bleeding time. In human beings this safety factor is not so great; normal plasma only contains about twice as much prothrombin as is needed to prevent the development of a hæmorrhagic tendency. The speed at which blood clots not only depends on the level of prothrombin

in the blood, but also upon its "convertibility." Increased convertibility of prothrombin may compensate for a relative deficiency in amount [42].

The liver is the site of prothrombin formation. Extensive hepatic damage in the human being is associated with hypoprothrombinemia [43], and in the experimental animal hepatic toxins, such as phosphorus, chloroform, carbon tetrachloride, hepatic tumours [374], and hepatectomy cause a fall in the blood prothrombin [44, 146]. If the hepatic injury is severe enough the administration of vitamin K is not effective in correcting the prothrombin deficiency [45, 146]. With regeneration of the liver, the blood prothrombin returns to normal levels. Chloroform, which is a well-known hepatic poison, produces a fall in the plasma prothrombin level of human beings [46]. Adrenaline on the other hand [191] causes a rise in the prothrombin level [191]. Nolf [110] has demonstrated the formation of prothrombin in the liver.

Busing and Zuzak [345] have shown that the complement titre in young chicks parallels the vitamin K intake. On a low intake the titre is consistently low; it rises with increased vitamin K intake and is higher in chicks receiving an excess of the vitamin than in normal birds.

The fate of plasma prothrombin after its formation in the liver and release into the circulating blood has been the subject of some recent experimental work [47]. Certain studies point to the lungs as the site of disappearance of plasma prothrombin. A possible explanation of the rôle of the lungs is thought to be the production of blood platelets in this organ [48]. Platelets undergo disintegration, and initiate the first stage of the clotting process by releasing thromboplastin, which, in the presence of calcium, changes prothrombin to thrombin.

Absorption of Vitamin K. Bile is essential for the proper absorption of natural vitamin K. Synthetic vitamin K analogues, particularly the water-soluble ones, do not need bile salts for their absorption, if given in adequate dosage (p. 811). The exact point of absorption in the gastrointestinal tract is not known for certain. Butt and Snell [179] believe that it is not absorbed through the colon or lower part of the ileum, but from the upper part of the small intestine. The taking of mineral oil (liquid paraffin) inhibits the proper utilization of vitamin K. The oil is not absorbed and dissolves the vitamin K, most of which is voided in the oil with the faeces [172, 190, 212, 213].

Storage of Vitamin K. Vitamin K is stored in the liver in small but definite amounts [49]. The storage cannot be appreciable because fatal hypoprothrombinemia can occur in a week.

Excretion of Vitamin K. Vitamin K is present in relatively large quantities in the faeces. It is unlikely that the total amount found is derived from the food by excretion into the intestine. Most of it is probably derived from the bacterial flora inhabiting the intestine. These are known to synthesize vitamin K, e.g., *E. coli* contains 750 to 1,600 Dam units per gram of dry weight. The formation of vitamin K by bacteria does not depend upon the presence of a provitamin, since it is also produced in a synthetic medium [272].

Pharmacology. No toxic effects have been observed clinically after

the administration of vitamin K or its analogues in therapeutic doses. Doses of 180 to 200 mg. of 2-methyl-1 : 4-naphthoquinone are well tolerated [148, 277]. Nausea and vomiting after large doses administered with bile salts have been reported, but this may have been due to the latter [148]. Forty milligrams of 2-amino-2-methyl-1-naphthol hydrochloride have been given intravenously for a period of nineteen days without any untoward effects [179], and 8 mg. of menaphthone have been administered daily for thirty months without untoward effects [277].

Excessive doses of vitamin K or its analogues are toxic to the experimental animal. The oral lethal doses (L.D.₅₀) of phthiocol and 2-methyl-1 : 4-naphthoquinone for mice are 200 and 500 mg. per kilogram respectively. The figures for the sodium salt of 2-methyl-1 : 4-naphthohydroquinone diphosphoric ester and 2-methyl-1 : 4-naphthohydroquinone sodium bisulphite are of the same order [275]. Vitamin K₁ is not lethal in doses of 25 mg. per kilogram [58]. The oral toxicities of the vitamin K analogues are approximately one-third to one-fifteenth of their subcutaneous toxicities. Chronic toxic effects are due to injury to the circulating red cells [58, 274], and aplastic anaemia [275]. In toxic doses the compounds produce respiratory depression and acute vascular congestion. As the maximum single therapeutic dose of any of the vitamin K analogues (*e.g.*, sodium salt of 2-methyl-1 : 4-naphthohydroquinone diphosphoric ester) appears to be about 20 mg. there is no danger of untoward reactions from doses of this order. The margin between the therapeutically effective and toxic dose is relatively enormous.

Russell and Page [214] have shown that 2-methyl-1 : 4-naphthoquinone is absorbed through the skin and that the application of 10 mg. of the substance in an ointment protects newborn infants from hypoprothrombinæmia. Vollmer and co-workers [276] have shown that the rate of percutaneous absorption is enhanced by decreasing the viscosity of the solvent. They find doses of 0.1 to 1 mg. effective in four hours. Fantl and Corkill [221] have administered 2-methyl-1 : 4-naphthoquinone percutaneously in cases of obstructive jaundice. This method of administration is not without danger as it may lead to dermatitis in certain individuals [215].

Menaphthone intramuscularly produces a lowering of the blood pressure in rats rendered hypertensive by wrapping both kidneys in silk [856]. In this respect it resembles the action of some other quinones.

If added to whole blood menaphthone causes marked methæmoglobin formation [357].

Massive doses of vitamin K, *e.g.*, 5 to 20 mg. per kg. produce hyperprothrombinæmia which is also produced by theophylline, theobromine and caffeine [880, 881]. As these drugs and aminophylline are widely used as vasodilators, it is questionable if they are safe as they might increase the tendency to thrombosis.

REQUIREMENTS OF VITAMIN K

The human requirements of vitamin K are not known. Bacterial synthesis of the vitamin can occur in the large intestine (p. 804), but

whether adult man needs an exogenous source is unknown. It is assumed that he does, as dietary vitamin K deficiency in man has been reported [52, 53]. According to Poncher [360] the vitamin K produced in the intestine by bacterial action is an important endogenous source in the human being, and is probably more important than exogenous sources. The newborn is considered to require an exogenous source as the prothrombin level falls after birth and only returns to normal levels after breast or artificial feeding. Considerable variations may occur in the vitamin K content of mothers' milk. Normally the newborn infant gets from almost nothing to 400 Dam units of vitamin K₁ (= 88 micrograms) from mothers' milk [298]. Hardwicke [299] found the minimal effective dose of a vitamin K analogue (tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphoric ester) necessary to prevent the development of hypoprothrombinæmia in the newborn infant. This was 0.5 to 5 micrograms daily. In established hypoprothrombinæmia of the newborn a dose of 1.25 mg. was sufficient to restore the prothrombin level to normal. Sells, Walker and Owen [88] consider that the vitamin K requirement of the newborn is 1 to 2 micrograms daily, an amount provided by the milk. This agrees with the figure of Hardwicke [299]. If adults require an exogenous source of vitamin K this would be something less than 0.1 mg. daily on the basis of the figures of Sells, Walker and Owen.

According to Mills, Cottingham and Mills [800] a rise in environmental temperature increases the requirements of vitamin K in the experimental animal. At any rate animals adapted to tropical heat seem more prone to severe manifestations of vitamin K deficiency than controls kept under temperate conditions. A survey of hospital statistics on hæmorrhage in the newborn shows that this is four times more prevalent among infants born in the Gulf States of America than among those born in northern states. This suggests that in infants at any rate a high environmental temperature increases the requirements of vitamin K.

VITAMIN K DEFICIENCY

Vitamin K deficiency, which is detected by a lowering of the blood prothrombin level (hypoprothrombinæmia), may occur in any of the following circumstances.

Inadequate Supply of Vitamin K. This may be due to four causes.

(a) *Nutritional Deficiency of Vitamin K.* That a nutritional vitamin K deficiency can exist is well supported by the experimental production of low prothrombin levels in chicks, rabbits, rats, and mice by the administration of diets deficient in vitamin K [54-56]. Apparently the vitamin formed by bacterial synthesis in the intestine is insufficient for normal requirements.

Kark and Lozner [52] observed four patients with a lowered blood prothrombin level unassociated with disease of the liver or biliary tract, and due apparently to a simple dietary deficiency. All patients had a history of dietary deficiency, three having eaten diets devoid of fruit and vegetables from one to nine years. Three had scurvy and one, an alcoholic, suffered from pellagra and subclinical scurvy. Vitamin K without bile

salts was given by mouth, and the day after the plasma prothrombin level returned to normal, showing that a nutritional deficiency of vitamin K was the cause of the hypoprothrombinæmia.

Scarborough [58] has reported eighteen cases of nutritional deficiency of vitamin K in patients without any clinical evidence of hepatic or biliary tract disease. An examination of the diet and laboratory tests showed that all patients were deficient in one or more vitamins.

Studies by Warner and his colleagues [164] on the prothrombin level of patients suffering from pellagra and multiple deficiencies revealed that vitamin K deficiency was not common. But complicating factors, such as prolonged diarrhœa, did at times, however, result in a serious deficiency of this vitamin. They believe that many chronically debilitated persons have a moderate hypoprothrombinæmia, which is probably due to inefficient utilization of vitamin K, rather than to a lack of it.

A deficiency of vitamin K has been recorded in anorexia nervosa, sufficient in fact to produce severe purpura [801].

(b) *Conditioned Deficiency of Vitamin K.* A conditioned deficiency of vitamin K may be produced by certain drugs. Thus sulphaguanidine and succinylsulphathiazole and other sulphonamides, such as sulphadiazine and sulphathiazole, administered to rats produce a fall in the blood prothrombin, probably by interfering with the bacterial synthesis of the vitamin in the gut of the animal [802]. The hypoprothrombinæmia can be prevented by vitamin K analogues [879]. This suggests that vitamin K be given to patients receiving sulphaguanidine or succinylsulphathiazole, particularly if submitted to any surgical procedures. Hæmorrhage responding to vitamin K has been recorded in patients given succinylsulphathiazole [806]. It is also stated that the prothrombin level is significantly decreased in patients taking sulphonamides [816].

According to Martin [359] menaphthone does not completely correct the fall in the prothrombin level produced by sulphaguanidine when added to purified diets; a mixture of menaphthone and vitamin C completely corrects the condition.

Huebner and Link [805] have shown that dicumarol, which produces a hypoprothrombinæmia, can be degraded to salicylic acid. It has been shown that salicylic acid and aspirin can induce hypoprothrombinæmia, which can be prevented by vitamin K [808, 804]. The possible clinical significance of the prothrombinopenic action of salicylates and aspirin has been discussed by clinicians [818], who suggest the use of vitamin K prophylactically to reduce the possibility of hæmorrhage in patients receiving large doses of salicylates. Meyer and Howard [804] suggest that the hæmorrhagic manifestations of acute rheumatic fever may be due to heavy dosage with salicylates. Small gastric hæmorrhages are known to occur after taking aspirin on an empty stomach. Shapiro [819] has shown that approximately 1 mg. of menaphthone (menadione U.S.P.) will counteract the prothrombinopenic activity of 1 gram of acetylsalicylic acid (aspirin) in patients receiving prolonged therapy with this drug.

(c) *Idiopathic Hypoprothrombinæmia.* Idiopathic hypoprothrombinæmia has been described [228, 807]. In one case described by Rhoads and Fitz-Hugh [228] the subject was an eighteen-year-old male with a hæmor-

rhagic diathesis, due to hypoprothrombinæmia. There was no evidence of dietary vitamin K deficiency. Giordano [307] has recorded a family incidence of idiopathic hypoprothrombinæmia with evidence that it may be associated with some functional liver defect. Plum [372] records idiopathic hypoprothrombinæmia in two otherwise healthy subjects that was refractory to treatment with vitamin K.

(d) *Hypoprothrombinæmia of the Newborn.* In the newborn infant and during the first few days of life there is a deficiency of prothrombin in the blood, and an increased tendency to hæmorrhage [57]. This subject is further discussed on p. 826.

Inadequate Intestinal Absorption. Inadequate intestinal absorption of vitamin K may result from :—

(a) Lack of bile in the intestine due to defective secretion of bile salts, as in infective jaundice, or due to biliary fistula, operations on the biliary tract.

(b) Obstructive jaundice of all types (*e.g.*, due to stones, cancer, stricture). Bile fails to reach the intestine, and ingested vitamin K is not absorbed.

(c) Pyloric and intestinal obstruction [68].

(d) **Pancreatic Insufficiency.** Pancreatic achylia and pancreatectomy, in the cat at any rate, are followed by a reduction in the prothrombin level [309]. Clinically a lowered prothrombin level is stated to occur in acute pancreatic disease [382]. It may be due to trypsin entering the blood stream and acting on the prothrombin and fibrinogen.

(e) Inadequate absorption due to various intestinal lesions and short-circuiting surgical procedures. Absorptive difficulties may result from excessive vomiting and loss of succus entericus from a drainage tube. In conditions associated with chronic and severe diarrhœa, food is hurried through the intestine, so that absorption of vitamin K, and of other vitamins for that matter, is impaired [59]. Hypoprothrombinæmia has been observed in cases of ulcerative colitis, regional enteritis, intestinal obstruction, Banti's disease, gastrocœlic fistula, intestinal neoplasm, polyposis coli, and those diseases in which fat absorption is particularly impaired, such as tropical sprue, idiopathic steatorrhœa, cœliac disease. Page and Bercovitz [810] found that nearly thirty per cent. of a group of patients with ulcerative colitis suffered from hypoprothrombinæmia. They consider that this may account for the bleeding from the rectum and mucous membranes ; this is reduced by giving vitamin K.

It has always been taught that bile is essential for the absorption of vitamin K. This view has been challenged since Kark and Souter [169] showed that fat soluble 2-methyl-1 : 4-naphthoquinone can be absorbed without the administration of bile salts even in complete biliary obstruction. Sanford and Shmigelsky [308] have also shown that in infants with an imperforate œsophagus and congenital absence of the gall bladder and bile ducts the plasma prothrombin may remain normal until death occurs. This contradicts the view that in infants vitamin K is synthesized from the food by bacterial action and that it requires bile salts for its absorption. This work is at variance with that of Macpherson [340], who fed infants with hydrocephalus glucose saline ; there was a continued fall in the

prothrombin level until death. Bile undoubtedly helps the absorption of vitamin K even if it is not essential. Mackie [60] investigated the vitamin K status of two hundred and seventy-seven miscellaneous medical and surgical cases by determining the prothrombin time of the plasma. In fifty-seven cases in which there was neither jaundice nor other evidence of hepatic disease, a lowered plasma prothrombin level was observed. Jaundice is therefore not a necessary concomitant of vitamin K deficiency.

It is probable that mineral oil (liquid paraffin) prevents the proper absorption of vitamin K [172, 212, 218].

Injury to the Liver. Even if bile is being produced and finds its way into the intestinal tract, prothrombin deficiency may still occur if the liver is damaged. The proper treatment of a jaundiced patient must therefore include restoration of liver function as well as vitamin K therapy.

Hepatic injury in the experimental animal by trauma or toxins causes a fall in the blood prothrombin level. The effect is noticeable in a few hours [233]. Clinically primary hepatic disease in man, such as cirrhosis acute yellow atrophy, chronic hepatitis and generalized carcinomatosis is frequently accompanied by hypoprothrombinæmia [61], and even after the administration of any anæsthetic in any major procedure the plasma prothrombin level also decreases [46]. Even manipulation of the liver at operation is said to lower the plasma prothrombin [166]. Borgström [817], who investigated the prothrombin level of a number of patients post-operatively, states that the most important factor affecting it is the anæsthetic. After spinal or local anæsthesia there is little change, but a significant fall occurs after operation. This work is questioned by Steggerda and Richards [811], who state that in the rat anæsthetic doses of chloroform and pentothal do not markedly affect the prothrombin level.

In rats primary hepatic tumours due to butter yellow (*p*-dimethylaminoazobenzene) cause hypoprothrombinæmia [874].

A fall in the prothrombin level is said to occur in patients given massive doses of arsenicals for the treatment of syphilis [812]. This is taken as evidence of liver damage by arsenicals. Elliott and co-workers [812] noted a hæmorrhagic encephalitis following massive arsenotherapy in syphilitics, but the prothrombin level of the blood was not recorded.

In a series of patients with liver disease, studied by Herbert [814] sixty-eight per cent. had hypoprothrombinæmia. If, however, the hepatic parenchyma is damaged or there is extensive liver disease, vitamin K is ineffective in restoring the prothrombin level. Herbert also states that patients with a normal plasma prothrombin level may develop hypoprothrombinæmia and bleed a few days after operation.

Infarction of the liver is associated with hypoprothrombinæmia [815].

Infection, particularly of the Respiratory Tract. There is increased destruction of prothrombin in artificial fever therapy, and prolonged fever, particularly pneumonia [167].

Tocantins and Hause [225] note that hypoprothrombinæmia is observed regularly in pneumonia, especially in the first stages of the disease. This diminution in prothrombin no doubt results from a disturbance in hepatic

function and may account for the delayed blood coagulability and the moderate hæmorrhagic manifestations observed in pneumonia. There are other evidences of liver dysfunction in pneumonia, such as hyper-urobilinuria and diminished response to liver function tests. The slowness of the return of the prothrombin level to normal after the acute stage of pneumonia and the poor response to vitamin K therapy are also evidence of liver dysfunction. Hypoprothrombinæmia in pneumonia may also be due to increased utilization of prothrombin in the fibrinous exudates of the lung.

A number of observers have recorded that patients suffering from active and chronic pulmonary tuberculosis generally show low prothrombin levels [226, 227, 287, 320-322]. Patients with active disease or hæmorrhage show a more marked diminution in the prothrombin level than chronic or healing cases. Hæmoptysis cannot, however, be predicted from the prothrombin level [320]. The prothrombin time in tuberculous patients is restored to normal by giving vitamin K, unless the condition has so progressed that there are signs of hepatic damage. Vitamin K has no effect on pulmonary hæmorrhage [235, 320]. These conclusions are contested by Plum and Poulsen [323], who state that the prothrombin level of tuberculous patients, even in those with hæmoptysis, is within the normal range.

As in the case of pneumonia the lowered prothrombin level is probably related to the toxæmia resulting from tuberculous infection.

Hæmorrhage. It is possible that the prothrombin level might fall after a massive internal hæmorrhage, particularly if fluids and not blood are given to the patient to restore blood volume. These would dilute the blood and some time might elapse before sufficient prothrombin is made in the liver. Coller and Farris [62] have recorded a case of massive gastric hæmorrhage in which a low prothrombin level was found after the intravenous administration of 7.5 litres of fluid.

It is stated that the prothrombin time is well below normal in patients during an attack of acute coronary occlusion [371].

TESTS FOR VITAMIN K DEFICIENCY

The most certain evidence of vitamin K deficiency is the presence of hæmorrhage in a patient with intense obstructive jaundice and in the newborn infant. Such cases call for immediate vitamin K therapy. Laboratory tests are also available so that vitamin K deficiency can be detected before hæmorrhage occurs. Vitamin K is not estimated as such, but the prothrombin level of the blood is found by a clotting method.

The prothrombin level should be determined as soon as possible after the blood is obtained from the patient, as the value increases with storage. Page and De Beer [824] have shown that at a given temperature the prothrombin time increases as a straight line function of the logarithm of the storage time; the rate of increase of prothrombin time is also directly proportional to the temperature at which the blood is kept.

Prothrombin Determination. Two-Stage Method. This method, originally designed in 1934 by Warner, Brinkhous and Smith [66], is

accurate but difficult to perform. In the first stage the prothrombin of the blood is converted to thrombin with an optimal amount of calcium and an excess of thromboplastic substance. In the second, or clotting stage, the amount of thrombin formed is measured by the time required for the clotting of a standard fibrinogen solution. It is assumed that the amount of thrombin is a measure of the amount of prothrombin originally present and that the clotting time depends upon the amount of thrombin. One unit of thrombin is defined as that amount which under specified conditions makes 1 c.c. of a fibrinogen solution clot in fifteen seconds. One unit of thrombin is formed from 1 unit of prothrombin. Normal human blood plasma contains approximately 800 units of prothrombin per cubic centimetre.

The two-stage technique is carried out by defibrinating oxalated plasma, which is then serially diluted and incubated with calcium chloride, a preparation of thromboplastin, and acacia. After this a standard fibrinogen solution is added and the clotting time determined. The dilution which will give a final concentration of 1 unit of prothrombin per cubic centimetre is determined, and the dilution is then an exact measure of the number of prothrombin units of the plasma. Thus normal human plasma must be diluted 800 times before it contains 1 unit of prothrombin per cubic centimetre. If a plasma contained 100 units of prothrombin units per cubic centimetre, the prothrombin concentration would be 88.8 per cent. of normal.

The method has been improved by Stewart and Rourke [67], and by Herbert [154], but the two-stage technique is complicated and does not lend itself readily to clinical procedure.

Prothrombin Determination. One-Stage Method. Two methods have been devised, one using oxalated plasma, the other whole blood.

Plasma Prothrombin Time. This was developed by Quick [68] and modified later by a number of investigators. Quick's method is a measurement of the time necessary for the conversion of prothrombin into thrombin and for the subsequent conversion of fibrinogen into fibrin by this thrombin. Briefly the method consists of determining the clotting time of oxalated plasma at 37.5° C. after the addition of an excess of a preparation of thromboplastin and a fixed amount of calcium chloride. With a given thromboplastin preparation the normal plasma prothrombin time is practically constant for any one species; for normal human beings it is between ten and twenty-five seconds. The thromboplastin preparation used by Quick was cephalin obtained from rabbit brain; others have used an extract of beef lung [178]. Most workers use dried extracts of brain. Owen and Toohey [198] simplify the process by using a saline extract. The prothrombin time for plasma containing as little as sixty per cent. prothrombin is nearly the same as for normal plasma, so that a normal prothrombin time does not necessarily mean that the plasma contains 100 per cent. prothrombin. This difficulty has been overcome by determining prothrombin times not only on whole plasma, but also after serial dilutions [52, 69].

Various modifications of the Quick method have been made. Thus Pohle and Stewart [70] studied the effect of adding varying amounts of

calcium chloride over a wide range and found that which gave the lowest clotting time. With optimal recalcification this was ten seconds for human plasma. Heparin has also been used to prevent coagulation instead of calcium chloride. For clinical purposes the use of tissue extracts as a source of thromboplastin has several disadvantages, since such extracts are tedious to prepare, their potency varies, and they may interfere with observations on the formation of the fibrin web when the plasma clots. Fullerton [71], Page and others [162, 192] have therefore used a preparation of Russell-viper venom (*e.g.*, Russven, Stypven) in place of other thromboplastin preparations [71, 162]. The addition of lecithin appears to potentiate the action of viper venom [72, 192]; the activity of the lecithin depends upon the presence of a fat soluble factor [241]. The one and two-stage methods do not give the same results, although if Russell viper venom is used in the one-stage method, the results of the two techniques are more in agreement [375].

Further modifications of the Quick method have been made by Holmboe [73], using a stable thromboplastic preparation from sheep brain, by Abramson and Weinstein [242] and by Reid [178], who uses lung extract as a source of thromboplastin. Reid employs a mechanical shaker for the plasma to increase the accuracy of the end point of the coagulation time.

Shapiro [325] and Brambel and Loker [326] have shown that greater sensitivity is obtained in the Quick method if the plasma is diluted 12.5 per cent.

A method utilizing a drop of blood in a loop coagulometer has been devised by Ulin and Barrows [328]. On rotating the loop the drop of blood moves readily on the wire; the clotting point is indicated by cessation of this movement.

Control determinations of prothrombin time are essential as Page and De Beer [329] have shown that periodic fluctuations may occur in the same individual over a period of a few weeks.

The *R-value* of Dam and Glavind [78] has also been used as an index of prothrombin activity. 2 c.c. of venous blood and 0.1 c.c. of a standard heparin solution (8 mg. in 2 c.c.) are centrifuged and the clotting power is determined by use of a thromboplastin preparation obtained from an extract of human brain. The concentration, K , of the brain extract which just coagulates the plasma in three minutes, when 1 drop of the extract is added to 4 drops of plasma, is determined. A corresponding determination is made on normal plasma (K_n). The value $R (= K/K_n)$ is a measure of the alteration in coagulation, while the reciprocal, $1/R (= K_n/K)$ is a measure of the ability to coagulate with thromboplastin. Within certain limits the ability of the blood to coagulate with the latter depends on the prothrombin content and is approximately proportional to the prothrombin concentration. In normal plasma $R = 1$. If $R = 5$, the prothrombin content is only 20 per cent. of normal.

Cheney [77, 265] used the *plasma coagulation time* as a simple test for vitamin K deficiency. Blood containing 10 mg. of oxalate per 5 c.c. is centrifuged at 3,500 revolutions a minute for five minutes and 0.2 c.c. of the resulting plasma pipetted into four tubes containing 0.5 c.c., 0.4 c.c., 0.2 c.c. and 0.1 c.c. of a 0.4 per cent. solution of calcium chloride

solution. The tubes are shaken briskly and the time noted for a clot to form after mixing the plasma and the calcium chloride. The shortest time recorded with the four tubes is the plasma coagulation time, while the longest is the plasma coagulation time with an excess of calcium, which is two to five times the optimal amount. Tests should be carried out preferably within thirty minutes of withdrawing blood. Cheney carried out this technique on 840 normal subjects and found that the average plasma coagulation time was 5.88 minutes, with a range of from two to eight minutes.

Blood and Serum Coagulation Times. Smith, Ziffren and their co-workers use whole blood instead of plasma [42, 76]. This method is suitable for the bedside. 0.1 c.c. of a standard thromboplastin solution prepared from rabbit or beef lung is placed in a small serological tube, which is filled with venous blood up to a 1 c.c. mark. The contents of the tube are mixed and the tube tilted every second or two to observe when clotting occurs. Clotting activity (per cent. of normal) =

$$\frac{\text{Clotting time of normal control}}{\text{Clotting time of patient}} \times 100$$

A modification of the Smith method has been made by Huber and Shrader [194] as a simple method for examining the coagulation time in the newborn.

Micro-methods have also been elaborated to overcome the objection of venipuncture, which may be difficult in the newborn. Karabin and Anderson [155] have devised a micro-modification of the Smith-Ziffren technique. Fiechter [74] withdraws 0.15 c.c. of blood from the tip of the finger, or the heel of the foot, or the lobe of the ear. One part of sterile M/10 sodium oxalate solution and 9 parts of blood are drawn up into a pipette graduated to 0.15 c.c. The oxalated blood is gently blown out on to a watch-glass incubated at 88° C. Then 0.15 c.c. of a solution of thromboplastin and finally 0.15 c.c. of a solution of M/40 calcium chloride are added. The coagulation time is calculated by noting the time elapsing between the addition of calcium chloride and the formation of a clot while the mixture is being gently agitated with a fine platinum loop.

Kato [75] employs 0.20 c.c. of blood, which is received into the hollow of a hanging drop slide, previously coated with 20 c.mm. of a two per cent. potassium ammonium oxalate solution, which has been allowed to dry. 10 c.mm. each of a freshly made thromboplastin suspension obtained from rabbit brain and of M/40 calcium chloride solution are mixed in the well of a hanging drop slide and 10 c.mm. of the oxalated blood added. The time is then noted for the formation of a gelatinous clot after the mixture has been agitated with a fine glass rod for five to six seconds. Improvements on this technique have been made by Innes and Davidson [162] and by Freudenberg [243], who uses human milk instead of brain extracts as a source of thromboplastin. Plum and Dam [827] employ a micro-method using only 0.1 c.c. of capillary blood.

A simple serum volume test has been proposed as a rough index of the bleeding tendency in jaundice [79]. This depends on the fact that in cases with a hæmorrhagic diathesis the serum volume is lowered. This

value is observed by allowing blood to stand for four hours in a graduated tube and removing the clot; the remaining volume of serum is noted. In the case of normal serum this is half the original volume of blood. The *serum volume index* is the serum volume over half the volume of blood withdrawn, the normal value is therefore 1. In cases with a considerable tendency to bleed it may fall as low as 0.1.

Ferguson [157] has compared the bleeding time (Ivy), serum volume index and Quick prothrombin time of blood as measures of vitamin K deficiency, and he finds that the first two, which are simple bedside procedures, compare favourably with the Quick method. Their correlation with the blood prothrombin time is close enough and their indication of a tendency to bleed accurate enough for the clinical detection of hypoprothrombinæmia.

Allen and Vermeulen [197] have pointed out that not only the prothrombin level, but the vitamin K reserves of the body are important in assessing a hæmorrhagic tendency. Unfortunately there is no method of measuring the vitamin K reserve. Apparently a patient may be at the point of depleting his reserve and still have a normal plasma prothrombin level.

CLINICAL MANIFESTATIONS OF HYPOPROTHROMBINÆMIA

A hæmorrhagic tendency due to a reduction of prothrombin only occurs when the level has fallen considerably. Quick [168] puts the critical level at fifteen to twenty per cent. of normal, although Kark and Souter [169] put it higher than this. They term the hæmorrhagic condition resulting from marked hypoprothrombinæmia hæmorrhagic hypoprothrombinæmia, and divide it into two classes, latent and spontaneous [170].

Latent hæmorrhagic hypoprothrombinæmia occurs at the sites of obvious trauma when the prothrombin level has fallen to about thirty-five per cent. of normal. Operation wounds begin to ooze or bleed, and the gums bleed if the teeth are vigorously brushed. Hæmatomata appear if the skin is pricked or a vein is punctured.

A spontaneous hæmorrhagic diathesis appears according to Kark when the blood prothrombin has fallen from fifteen to twenty per cent. of normal, and is seen particularly in the newborn, in idiopathic steatorrhœa, obstructive jaundice and severe parenchymatous hepatic disease. Large hæmatomata may appear on the back, thighs and other pressure areas, and hæmarthrosis, hæmatemesis, epistaxis, hæmaturia or melæna may occur. Menorrhagia, retinal hæmorrhage and in the case of infants intracranial hæmorrhage have been noted. Intractable hæmorrhage from trivial wounds and in infants umbilical hæmorrhage have been described. The blood coagulation time is prolonged but capillary fragility is unaltered.

VITAMIN K THERAPY

The need for treatment with vitamin K depends on the clinical detection of vitamin K deficiency or on the estimation of the prothrombin time. A patient with a prothrombin time (Quick) of more than thirty seconds,

the normal value being ten to twenty-five seconds, demands careful observation, and he may be considered a potential case of hæmorrhage if his prothrombin time is above forty-five seconds [179].

Vitamin K concentrates obtained from alfalfa or from cereal grass (*e.g.*, klotogen, cerophyl) were used originally, but the dosage required to give the best prothrombin response was not studied in any systematic way. The dosage was of the order 20,000 to 50,000 Dam units with 1 to 4 grams of bile salts or sodium desoxycholate, although massive doses of 100,000 to 500,000 units were given in some cases [81-85]. In spite of these large doses it appears that 20,000 Dam units of vitamin K₁ a day is sufficient to maintain normal clotting time.

The introduction of vitamin K analogues has completely replaced the use of the natural vitamin K₁ concentrates. The first of these was phthiocol, used in a dosage of 25 to 100 mg. [86, 188]. This has been replaced by 2-methyl-1 : 4-naphthoquinone and its derivatives.

2-Methyl-1 : 4-naphthoquinone (menaphthone, menadione) is the most commonly used analogue. The dosage employed is from 1 to 10 mg. orally or intramuscularly, although 2 mg. daily [232] orally or parenterally every few days [228, 381] is probably effective in most cases. In the case of infants with hypoprothrombinæmia 1 mg. parenterally or orally is sufficient. Menaphthone is said to be more effective by mouth in an oily medium than in tablet form or as an aqueous suspension. When given parenterally a rise of fifteen to sixty per cent. of the plasma prothrombin occurs in from two to eight hours and is maintained for several days [228]. As it is not water-soluble the solution for injection is in oil. This is given intramuscularly not intravenously. Menaphthone can be prepared in a form suitable for intravenous injection by dissolving 10 mg. in 2 c.c. of alcohol and slowly introducing the solution under the surface of a large volume of sterile ten per cent. glucose solution [230].

If water-soluble derivatives are given intravenously hæmorrhage can be controlled within one and a half to three hours after injection, while the blood prothrombin level rises rapidly and reaches a normal level in twenty-four to forty-eight hours [169]. A single intravenous injection, however, will not maintain a normal level, and the prothrombin will fall again. A single intramuscular injection of an oil soluble analogue, such as 2-methyl-1 : 4-naphthoquinone, while not so effective immediately for the control of hæmorrhage, will maintain the prothrombin level for some days. Hence in the case of a patient with active hæmorrhage and a lowered prothrombin level an intravenous injection of a water-soluble preparation should be given, followed by intramuscular or oral administration of a fat-soluble derivative. Kark and Souter [169] give 2 to 6 mg. of 2-methyl-1 : 4-naphthohydroquinone-8-sodium sulphonate in actively bleeding cases with a normal liver.

When vitamin K was first used clinically it was given to patients with diseases of the liver and biliary tract suffering from hypoprothrombinæmia, and usually administered in an oily medium with bile salts or bile given by a duodenal tube. Until recently 2-methyl-1 : 4-naphthoquinone was always given orally with 0.8 to 2 grams of bile salts, but Kark and Souter [169] have shown that this compound in tablet form is effective

orally in doses of 2 mg. three times a day without bile salts even in patients with complete biliary obstruction. They found doses of 1 mg. effective in some patients with idiopathic steatorrhœa. Olwin [229] states that even 0.1 mg. of 2-methyl-1 : 4-naphthoquinone is effective in some cases. Morse and Schmidt [171] have also shown that the fat soluble vitamin K analogues, such as phthiocol and 2-methyl-1 : 4-naphthohydroquinone are absorbed from the intestinal tract of rats with bile fistulæ even if bile salts are not administered. Stewart [174] states that the effectiveness of

Compound.	Dosage.	Route.	Remarks.	References.
Vitamin K ₁ oxide . . .	10 mg.	Intramuscular or intravenous.	Solubilized by adding 10 mg. in 3 c.c. alcohol to 10 c.c. saline.	268, 284
Menaphthone, menadione	1-10 mg.	Oral and intramuscular.	Prothrombin time returns to normal in 8-36 hours, begins to rise in a few hrs.	65, 87, 107, 188, 189, 140, 144, 148, 158, 168, 169, 170, 178-184, 207, 245, 255, 257, 259, 332.
2-Methyl-1 : 4-naphthohydroquinone dibutyrate.	10-20 mg.	Oral	—	—
Menadione bisulphite (2-methyl-1 : 4-naphthohydroquinone bisulphite ; 2-methyl-1 : 4-naphthohydroquinone-8 sodium sulphonate).	1-10 mg.	Oral, intramuscular, intravenous.	Acts parenterally in a few hours.	89, 107, 149, 169, 174, 179, 280, 281, 246.
4-Amino-2-methylnaphthol hydrochloride.	1- 6 mg.	Intravenous and oral.	Effective in 2 hrs. Prothrombin normal in 24 hrs.	65, 188, 150, 175, 179, 185, 280, 344.
1 : 4-Dihydroxy-2-methyl-3 naphthaldehyde.	10-25 mg.	Intravenous and oral.	Given up to 200 mg. orally.	188, 143, 148.
2-Methyl-1 : 4-naphthohydroquinone disuccinate	5-10 mg.	Parenteral and oral.	Solution unstable. Must therefore be freshly prepared.	182, 178.
2-Methyl-1 : 4-dipropionyl naphthohydroquinone.	1- 4 mg.	Oral and parenteral.	Response in 24 hrs.	174.
Tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphoric acid ester.	5-20 mg.	Oral, i.m. i.v.	One of most active of water-soluble analogues	280, 281, 237, 256, 261, 262, 333, 334, 336, 337.

2-methyl-1 : 4-naphthoquinone is increased by taking desoxycholic acid or bile salts.

The treatment of hypoprothrombinæmia with just sufficient vitamin K to raise the prothrombin level of the blood to normal will not ensure against hæmorrhage except for very short periods. Treatment must be continued beyond this point to build up a reserve in the body before operation and the administration of the vitamin continued post-operatively. Unless this is done the prothrombin level will fall when treatment is withdrawn, with danger of subsequent hæmorrhage.

There appears to be no danger of hyperprothrombinæmia, *i.e.*, a prothrombin level higher than normal, through overdosage with vitamin K

or its analogues. Stewart [282] records only three cases in which the plasma prothrombin was raised to more than 110 per cent. of normal by vitamin K; one patient was dehydrated and the other two suffered from recurrent thromboses of peripheral veins. He has given up to ten times the effective dose of vitamin K without untoward effects and without elevating the plasma prothrombin above normal.

Hyperprothrombinæmia has been recorded in untreated acute thrombophlebitis, in the initial stage of embolism, and in multiple myeloma [880].

The table on p. 819 gives the various vitamin K analogues with range of dosage. In calculating dosage daily determinations of the prothrombin level should be made and dosage adjusted to maintain this at a normal level.

CLINICAL USES OF VITAMIN K

In Conditions not Associated with Jaundice or Liver Disease. It may be said at the outset that vitamin K cannot be used merely to check bleeding, irrespective of its origin, nor has it any appreciable effect on the prothrombin level of subjects not suffering from hypoprothrombinæmia [267].* It does not appear to be of value in hæmophilia, purpura, and diseases of the blood-forming organs; although there is a tendency to bleed in these conditions there is no deficiency of prothrombin. Nor is the vitamin effective for the control of hæmorrhage in the normal individual. The chief indications have been for the prevention and treatment of hæmorrhage associated with jaundice and liver damage (cholæmic hæmorrhage) and the hæmorrhagic diathesis of the newborn.

It is now being realized that prothrombin deficiency may be encountered in other conditions, particularly those associated with an abnormal state of the gastro-intestinal mucosa, which interfere with the absorption of the vitamin. Patients with gastro-intestinal disease also consume diets poor in vitamins. Thus Snell [82, 88, 188] and his co-workers have reported occasional instances of vitamin K deficiency in ulcerative colitis, polyposis of the colon, regional ileitis, pyloric obstruction, sprue and gastro-jejuno-colic fistula. Bercovitz and Page [810] have shown that some thirty per cent. of patients with ulcerative colitis have a lowered prothrombin level, and that the administration of vitamin K analogues percutaneously (1 gm. daily) or orally (2 mg. daily) causes a marked diminution in the mucous membrane bleeding seen in this condition.

The plasma prothrombin levels of thirteen patients suffering from intestinal lesions were examined by Butt, Snell and their co-workers [82, 94]. The cases included ulceration, external and internal fistulæ, intestinal obstruction, diarrhoea and collapse of the ileum. A marked hypoprothrombinæmia was observed in many of the patients, and in three hæmorrhage occurred. The vitamin K deficiency in these patients was considered to be due to an insufficient amount of normal intestinal mucosa for adequate absorption. Butt and Snell suggest that the blood

* Palladin [378] claims that menaphthone is effective in controlling hæmorrhage, even when not associated with hypoprothrombinæmia. He gives no details or controlled observations.

prothrombin should be determined as a prelude to therapy if bleeding occurs in any medical or surgical case in which a diminution of the absorptive surface of the intestine is probable or suspected, or in which biliary obstruction or infection is present. Although there may not be spontaneous hæmorrhage before, if patients with a low prothrombin level are submitted to surgical procedures the hæmorrhage may become serious.

Fanconi [89] reported on six patients with sprue who had a bleeding tendency and a prolonged coagulation time. Dam and Glavind [54], however, found normal values in their cases. Others have found a moderate hypoprothrombinæmia, but the tendency to a hæmorrhagic diathesis in this disease is rare [82, 94]. It has been described by Kark and his colleagues [169] and by Allen [176]. Engel [92], Koller and Wuhrmann [195] used vitamin K successfully in cases of sprue with a bleeding tendency. Huet [95] gave vitamin K orally to a patient suffering from sprue and showing an R-value (p. 815) of 6.5 to 8.5. This was reduced to normal levels by vitamin K, but it returned to its former high value when the administration of vitamin K was discontinued.

Others have recorded low prothrombin levels in severe diarrhoea, intestinal obstruction, multiple strictures, fistulæ, mesenteric obstruction hæmorrhagic retinitis, and in pseudo-hæmophilia hepatica of childhood [89]. Stewart [196] and his colleagues have reported prothrombin deficiency in patients with peptic ulcer, malnutrition and cachexia. Mackie [98] and his co-workers investigated the prothrombin time (Quick) of two hundred and seventy-seven miscellaneous medical and surgical cases, and observed an elevated prothrombin time in fifty-seven cases in which there was no evidence of jaundice or hepatic insufficiency. These cases were distributed among eighteen different conditions as follows:—

Chronic ulcerative colitis	28	Chronic hæmorrhagic diathesis	1
Peptic ulcer	9	Lung abscess	1
Regional enteritis	3	Cardiac insufficiency	1
Lobar pneumonia	3	Pernicious anæmia	1
Gastritis	1	Retroverted uterus	1
Carcinoma of stomach	1	Hypothyroidism	1
Acute appendicitis	1	Dietary insufficiency	1
Carcinoma of the rectum	1	Sprue	1
Postpartum hæmorrhage	1	Banti's disease	1

According to Warner and Owen [264], patients with pernicious anæmia show prothrombin levels of only forty to sixty per cent. of normal; the hypoprothrombinæmia is not rectified by vitamin K therapy, unless liver is given simultaneously.

Moderate reduction in the prothrombin level in such diverse conditions as duodenal ulcer, melæna, lung abscess and rectal carcinoma has been observed by Stewart and Rourke [96]. Treatment with vitamin K and cholic acid resulted in a gradual rise in the prothrombin level. Cases of hypoprothrombinæmia following acute appendicitis with perforation have been described, and it has been suggested that the prothrombin concentration should be determined in cases of peritoneal infection of gastrointestinal origin [156].

Rawls [234] has also observed a hypoprothrombinæmia in patients

suffering from rheumatoid arthritis, hepatitis due to cincophen, hæmorrhage from gastric ulcer, myelogenous leukæmia, aplastic anæmia, thrombocytopenia, hyperthyroidism, malignant endocarditis, chronic intestinal diseases, and toxic reactions due to the administration of gold. In most of these the prothrombin level returned to normal after administering vitamin K. Thordarson [885] noted a low prothrombin level in ten out of fourteen patients with myeloid leukæmia. The administration of vitamin K₁, however, failed to raise it to normal.

An unidentified non-saponifiable, fat-soluble substance has been prepared from liver, which is effective in controlling excessive uterine bleeding, and it has been suggested that this might be vitamin K or a substance resembling it [97].

This has not been confirmed. According to Gubner and Ungerleider [886] and Dietz [90] tetrasodium 2-methyl-1:4-naphthohydroquinone diphosphoric acid ester is effective in the treatment of menorrhagia in doses of 5 to 10 mg. orally or intramuscularly taken over a period of five days. The former report that an excessive flow is reduced and that dysmenorrhœa due to clot formation is diminished. It has been shown by Bonilla [91], however, that in over three hundred gynæcological cases the prothrombin time was within normal limits. If this is so, there is no scientific basis for the use of vitamin K in gynæcological hæmorrhagic conditions.

Vitamin K has failed to control pulmonary hæmorrhage (p. 887).

In Jaundice and Liver Disease. Incidence. The hæmorrhagic tendency in patients suffering from obstructive jaundice and diseases of the liver has long been known, and constitutes a distinct hazard in the case of patients submitted to operation. Thus in nearly four thousand operations on jaundiced patients sixty-one of the four hundred and forty-two post-operative deaths (18·8 per cent.) were attributed to hæmorrhage [104], a figure which agrees closely with the twelve per cent. given by Sir John Fraser [105]. A much higher figure is given by Butt [82]. Before it was realized that such hæmorrhage was due to a vitamin K deficiency, conditioned by lack or escape of bile salts, the patient was prepared for operation by giving large doses of glucose and by calcium therapy. A review of the older literature reveals that the incidence of bleeding in patients with obstructive jaundice was higher before the development of modern pre-operative and post-operative care. Absence of bile salts in the intestine may occur without obstruction of the common bile duct, as it has repeatedly been shown that bile from the liver following obstruction or severe liver damage may contain no bile salts. All patients with obstructive jaundice should have pre-operative prothrombin determinations and operation deferred if possible until the prothrombin level is raised to normal. The value of vitamin D in preventing hæmorrhage in jaundiced patients is discussed on p. 647.

Causes. Suggested causes for this post-operative fall in the prothrombin level are: (1) increased utilization of prothrombin for the formation of exudates, (2) defective absorption of vitamin K due to deficient secretion of bile salts, (8) liver damage resulting from anæsthesia (p. 812), infection and operative trauma, (4) lack of adequate vitamin K reserves.

Prothrombin Level. The first studies of plasma prothrombin in patients with obstructive jaundice were made by Quick in 1935 [99]. Shortly afterwards, in 1936, Hawkins and Brinkhous [100] found low prothrombin levels in dogs with chronic biliary fistulæ, and they found that the levels could be raised to normal by giving bile salts and vitamin K. Two months later Warner, Brinkhous and Smith [80, 101] reported the first successful treatment of patients with a hæmorrhagic tendency associated with hypoprothrombinæmia and obstructive jaundice. This was followed by similar reports from the Mayo Clinic [82], Copenhagen [102], and other centres [12, 67, 80, 103]. The prothrombin level at which cholæmic hæmorrhage occurs has been studied in a number of human patients. Brinkhous and his co-workers [80] found that the bleeding occurred when the prothrombin level was below thirty-five per cent., but subsequent studies have shown that no sharp prothrombin level can be considered as a bleeding level, as some patients with a plasma prothrombin as low as twenty per cent. of normal may show no evidence of hæmorrhage. Other factors, such as trauma, infection, operative hæmorrhage and exudate formation play a part in determining the prothrombin level at which hæmorrhage occurs. Stewart and Rourke [96] found that massive hæmorrhage occurred in six jaundiced patients at prothrombin levels varying from thirteen to thirty-six per cent. of normal; on the other hand hæmorrhage has been observed in patients with prothrombin times varying from normal to twice the normal value [82]. The reported differences in the incidence of hypoprothrombinæmia may be due to the method used for estimating prothrombin.

Post-operative Hypoprothrombinæmia. It has long been recognized that the hæmorrhagic tendency in obstructive jaundice often occurs within the first few days after operation. Little evidence of excessive bleeding is seen at operation, but massive and even fatal hæmorrhage may occur a few days later. Butt, Snell and Osterberg [82a] mention the occurrence of prolonged prothrombin times and post-operative hæmorrhage in several patients, in all of whom hæmorrhage first occurred between the first and fourth day post-operatively. The common occurrence of post-operative cholæmic bleeding led to a study of prothrombin levels during this period. Butt, Snell and Osterberg [82, 82a, 103] observed that the prothrombin time was often prolonged in jaundiced patients following operation, and that those patients suffering from considerable liver damage as well as from obstructive jaundice were most susceptible to post-operative hæmorrhage. Stewart [106] and his colleagues have confirmed this, even after blood transfusions given routinely at the time of operation. They found that the fall in the prothrombin level averaged twenty to twenty-five per cent., the minimum level being reached between the first and fourth days, thus coinciding with the period when hæmorrhage most commonly occurs. Brinkhous [80] also observed in one case that after operation the prothrombin fell from a nearly normal level to twenty per cent. of normal by the tenth day. This was attributed to bile draining away externally.

It has been suggested that anæsthetics might contribute to the post-operative hypoprothrombinæmia in patients with jaundice or disease of the biliary tract. Although chloroform produces a hypoprothrombinæmia, Allen and Livingstone [200] have concluded from prothrombin

studies on a hundred and six patients who underwent operations under ether, vinesthene, nitrous oxide, ethylene, bromethol (avertin), nupercaine and spinal and local anaesthesia, that these anaesthetics have no effect on the prothrombin level. They suggest that some form of storage of vitamin K or prothrombin occurs in the body and that failure to replenish this store in the patient with obstructive jaundice or biliary fistula accounts for the post-operative hypoprothrombinæmia.

For many years it has been noted that there is liver impairment in hyperthyroidism. Andrus and Lord [228] have shown that there is an immediate post-operative fall in the plasma prothrombin level, averaging fifteen per cent. and amounting to forty per cent. or more in some cases. The values were similar to those obtained after chloroform anaesthesia (p. 823). It would, therefore, seem advisable to improve the hepatic function of the hyperthyroid patient pre- and post-operatively.

Treatment. Vitamin K should be given when the prothrombin level is lower than seventy per cent. of normal. A number of workers consider seventy-five to eighty per cent. a safe pre-operative level. Herbert [814] states that post-operative hæmorrhage is likely to occur in patients with hepatic disease if the prothrombin level falls below sixty per cent. of normal. She has also shown that a normal prothrombin level before operation may be followed by hypoprothrombinæmia and hæmorrhage after a few days. Routine administration of vitamin K does not as yet avoid the necessity for prothrombin determinations, as the response to the administration of vitamin K is not uniform. If a rapid response is required one of the water-soluble derivatives, such as the phosphate or bisulphite (pp. 801, 818) may be given intravenously. Allen [197] suggests that all cases of jaundice and biliary fistula should have vitamin K pre-operatively even if the prothrombin level is normal, as the body stores may be virtually depleted of the vitamin. It has been emphasized that vitamin K therapy should be continued during the post-operative period, especially if bile continues to drain from the wound. In addition to lack of the vitamin, the patient no doubt uses prothrombin in large amounts in the formation of fibrinous exudate in the margins of the wound. Too much cannot be expected of vitamin K therapy. The plasma prothrombin level depends on the functional capacity of the liver as well as upon the absorption of vitamin K from the intestine. There is general agreement that vitamin K therapy is ineffective in raising the prothrombin level in cases of hepatic damage. Thus the administration of the vitamin has failed to restore normal prothrombin levels in cases of cirrhosis of the liver, acute yellow atrophy, Wilson's disease, Banti's syndrome, multiple liver abscesses, fatty infiltration and infarction of the liver, acute hepatitis and carcinoma of the liver [107, 144, 814, 815]. In these conditions there is damage to the hepatic parenchyma.

Blood transfusion may be necessary if severe loss of blood has occurred in a jaundiced patient, but in the absence of vitamin K this will only control hæmorrhage as long as the prothrombin of the transfused blood lasts (six to twelve hours). The use of stored or "bank" blood is unsatisfactory since it is low in prothrombin [109, 219], although it is said that when the blood is taken from the donor the fall in the prothrombin level can be

prevented by drawing off the plasma and adding citric acid solution [268].

Kinsey [888] gave vitamin K to five cases of acute yellow atrophy of the liver with the plasma prothrombin reduced to such a low level that hæmorrhages occurred. No response was obtained. He, therefore, gave blood transfusions to the patients after giving the donor 6 to 10 mg. of menaphthone intramuscularly twenty-four hours before transfusion. In three cases the hæmorrhages were temporarily controlled. Kinsey suggests that vitamin K stimulates the formation of prothrombin or one of its precursors in the blood of the donor in abnormally large amounts. This is unlikely as vitamin K has no effect on the prothrombin level if this is already normal.

Post-operative Treatment. Prothrombin determinations on patients with jaundice or liver damage should always be carried out daily for the first four days after operation, and every other day for at least eight or ten days longer, since hæmorrhage has been observed as late as the eighteenth day after operation. An increase in the prothrombin time should be considered an indication for vitamin K therapy. Patients whose prothrombin time before surgical treatment has been high should receive vitamin K for several days after operation. Those with prothrombin times of more than thirty seconds should be treated with special care, and those with times of more than forty-five seconds must be considered as potential bleeders. In surgical procedures in general practice there is no objection to the routine use of vitamin K without prothrombin estimations, since the vitamin is inexpensive and harmless in therapeutic doses.

It has been learnt from experience that a normal prothrombin time on the day of operation is no guarantee that post-operative hæmorrhage will not occur. This is particularly true of cases of subacute and chronic cholecystitis, who may come to operation and not be jaundiced. Some degree of liver damage may be present and the danger of post-operative hæmorrhage may be real.

Liver Function Test using Vitamin K. Attempts have been made to use the prothrombin response to vitamin K as a test of liver function, since hypoprothrombinæmia associated with severe liver damage does not respond to treatment with vitamin K [186, 198, 266]. Lucia and Aggeler [165], however, found that although hypoprothrombinæmia in cases of acute diseases of the liver was often refractory to treatment with vitamin K, no significant correlation existed between the hippuric acid function test and the plasma prothrombin, either before or after administering vitamin K. This was confirmed by Kark [201], White [220] and their colleagues. Moreover, patients with severe liver impairment, as demonstrated by the hippuric acid test, may have normal prothrombin levels. The fact remains, however, that in acute diseases of the liver, such as acute hepatitis and acute yellow atrophy, and chronic diseases such as portal cirrhosis, there is generally a low prothrombin level which is usually not raised after administering vitamin K.

Lord and Andrus [228] and Allen and Julian [887] have suggested that the response of plasma prothrombin to intramuscular injections of 2-methyl-1 : 4-naphthoquinone may be used to differentiate between intra-hepatic and extrahepatic jaundice. Lord and Andrus found that there

was a rise of from ten to sixty-two per cent. in the plasma prothrombin following the intramuscular injection of 2-methyl-1 : 4-naphthoquinone in extrahepatic jaundice (common duct stone, cholangitis, carcinoma of the head of the pancreas, stricture of the common duct), whereas in cases of intrahepatic jaundice the response was ten per cent. or less. The cases of extrahepatic jaundice were confirmed at operation or autopsy. Kark and Souter [224] also observe that in patients with intense jaundice the return of a low prothrombin level to normal after vitamin K therapy favours a diagnosis of obstructive or extrahepatic jaundice. They recognize five types of response to vitamin K therapy in patients with liver disease : (1) Rapid response in obstructive jaundice ; (2) no response in gross liver disease ; (3) partial response, with the level still slightly subnormal after vitamin K therapy in cases of acute or subacute parenchymatous hepatic damage of a moderate degree ; (4) a gradual rise in the prothrombin level with treatment, coincident with clinical improvement, in cases of infective cholangitis, catarrhal jaundice, acute or toxic hepatosis and obstructive jaundice complicated by infection ; (5) a fluctuating prothrombin level, above the threshold for hæmorrhage, in patients with chronic and long-standing hepatic disease, usually unassociated with jaundice.

Allen [384], in a critical review of liver function tests, states that the prothrombin response to vitamin K is as good as the bromsulphthalein excretion test or the serum albumin-globulin ratio for detecting liver damage. In the jaundiced patient, where the diagnosis lies between obstructive and intrahepatic jaundice, the character of the prothrombin response to vitamin K may enable a diagnosis to be made, when all other methods, short of surgery, have failed. Allen gives the following conditions for performing the prothrombin response test. There must be a significant prothrombin reduction (less than seventy-five per cent.) ; the patient must be eating well and afebrile ; a sensitive prothrombin method should be used ; and the vitamin K must be given parenterally as a water-soluble analogue in two doses of 10 mg. several hours apart. In obstructive jaundice there is at least a twenty-five per cent. increase in the prothrombin, whereas in cases of extensive liver damage there is little or no response. These observations have been confirmed by Stein [383], who claims to have achieved an accuracy of over ninety-five per cent. in differentiating between intra- and extra-hepatic jaundice using the prothrombin response to vitamin K test. Of course the interpretation of the test may be difficult in cases of combined intra- and extra-hepatic jaundice, *e.g.*, carcinoma of the liver with metastases in the portal glands, and biliary cirrhosis following chronic obstruction of the bile ducts.

Vitamin K in Hæmorrhagic Diseases of the Newborn. **Prothrombin Levels.** Low and variable plasma prothrombin levels in the newborn and in early infancy, and the existence of a hypoprothrombinæmia in cases of hæmorrhagic disease of the newborn were observed by Brinkhous [111] and his colleagues. They noted that blood from the umbilical cord showed prothrombin values of from fourteen to thirty-nine per cent. of the normal adult figure. Values of ten per cent. have been observed [120]. During the first eleven days of life values ranging from twenty-six to forty-four per cent of normal were observed, and a study of later age groups showed a gradual

rise in the prothrombin level until it became almost normal at ten months. This work has been confirmed by a number of investigators. Hellman and Shettles [112] determined the prothrombin content of the blood of newborn infants and their mothers; the values were 22.2 and 102.5 units respectively. In the case of premature infants it was as low as 8.8 units. These extremely low infant prothrombin levels were raised from sixty-eight to one hundred and thirty per cent. of normal values by giving vitamin K to the mother just before delivery, or directly to the infant after birth.

Waddell [118] and his associates have made two thousand two hundred and thirty-two prothrombin and clotting time determinations on one hundred and ninety-one control infants and one hundred and eighty-one infants treated with vitamin K. They find that the period of most marked deficiency occurs from forty-eight to seventy-two hours after birth; a forty-eight hour peak was also observed in premature infants. There is also an apparent seasonal variation in the incidence of prothrombin deficiency in the newborn [118, 194, 341]. A period of marked deficiency occurs during the winter months, reaching a peak in March. This has been confirmed by Snelling [344] and Lehmann [341]. This seasonal variation may be an expression of a lack of vitamin K in the diet of the pregnant woman during the winter season. Waddell, from a statistical analysis of the death rates from birth injury over a period of fourteen years, finds that the preponderance of deaths occurs in the winter months, and he points out that the peak periods correspond to the months in which prothrombin deficiency is at its highest. He concludes that the winter peak deaths are expressions of prothrombin deficiency aided by trauma.

Bray and Kelly [114] state that the prothrombin time of the newborn is very variable, tending normally to be within the adult range on the first day, to rise to a peak, definitely higher than the normal adult range between the second and fifth days, usually on the third day, and to fall again within the adult range after the fifth day. These observations agree with those of Waddell and Guerry [115], who observed that a prolongation of the prothrombin time often occurred between the first and sixth days of life. It is said that circumcision was delayed under the Mosaic law until the eighth day because of the risk of bleeding if done earlier. Huber and Shrader [194] found a return to normal at the beginning of the second week. The administration of vitamin K or an analogue within twenty-four hours of birth prevents the fall in the prothrombin level [208]. The prothrombin time is always high in hæmorrhagic disease of the newborn, but it may also be high without any hæmorrhagic tendency. For example, prothrombin levels as low as ten to fifteen per cent. of the adult normal level were observed in several infants without evidence of hæmorrhagic disease [120].

The prothrombin times of a hundred and seventy-three newborn infants, both mature and immature, were determined by Kato and Poncher [116], using the former's micro-method (p. 816). The average prothrombin time of a hundred mature infants on the first day of life was forty-three seconds, gradually shortening as the infants grew older, until by the tenth day it was twenty-five seconds; the average pro-

thrombin time of seventy-three premature infants was slightly higher—46.5 seconds. In their studies on the clotting time in the newborn Dam, Tage-Hansen and Plum [117] observed an increase in the R-values, 1.8 to 8.0, from the first to the sixth day of life. Studies on the cord blood of newborn infants showed prothrombin levels of twenty-three to sixty-five per cent. of normal [120, 147].

Cause of Hypoprothrombinæmia of the Newborn. Plum, Dam and Thordarson state that the prothrombin level of the mother just before birth is higher than that of the normal non-pregnant woman [141]. This suggests that since the newborn has a low prothrombin level the permeability of the placenta to vitamin K must alter towards the end of pregnancy, preventing it from passing in sufficient quantities from the mother into the blood of the fœtus. On the other hand, Javert and Macri [252], from a study of 200 pregnant women, observed that the prothrombin level was below seventy per cent. of normal in a number of them; the average level was eighty-nine per cent. The cord blood prothrombin at delivery was twenty-four per cent. of normal, confirming the view that the prothrombin molecule does not readily traverse the placental barrier. Vitamin K analogues given to the mother pass through the placenta to the infant [185].

The hypoprothrombinæmia of the newborn may be due to the fact that the intake of food in the first twenty-four hours is low and that milk, particularly from a mother on an inadequate diet, is a poor source of vitamin K. The gut of the newborn is also sterile, so that bacterial synthesis of the vitamin is not possible. This might explain the observation of Javert [118] that hæmorrhage in the newborn is twice as common in babies born in the New York Hospital, where feeding is conducted under the most sanitary conditions, as in babies born at home. Salmonsén and Nygaard [121] observed that supplementary feeds of cow's milk in addition to breast milk prevents the normal fall in the prothrombin level. Gellis and Lyon [249] have also noted that the type of feeding of the newborn influences the prothrombin level. They observed that the greatest fall in the prothrombin level and the most prolonged recovery to normal levels occurred in infants breast-fed every four hours; the average prothrombin level after five days was 61.2. The level in a comparable group receiving breast milk and supplements of evaporated milk and corn syrup was 95.4 at the end of the same period. Another factor contributing to the hypoprothrombinæmia of the newborn may be the small amount of bile secreted by the liver.

Macpherson [840] has produced evidence to show that hæmorrhagic disease of the newborn is due to a dietary deficiency of vitamin K in the mother during pregnancy. The mean prothrombin level of the babies of twenty-four mothers with an adequate dietary history was eighty per cent. at birth and sixty-five per cent. in three days; the average prothrombin level at birth of nine babies of mothers giving a history of gross dietary deficiency in pregnancy was under forty per cent. and remained low for a long time. There were signs of cerebral irritation in three and in two necropsy revealed that hæmorrhage took place on the day after birth. In addition there were six cases of hæmorrhagic disease. Pregnancy toxæmia

in the mother, prolonged labour and prematurity had little effect on the prothrombin level in the infant. But in cases of prolonged labour in which more than one inhalation anæsthetic was given the prothrombin level of the baby was abnormally low, and three out of four babies had severe cerebral symptoms and two had manifest hæmorrhagic disease. Macpherson attributes these low values to chloroform producing liver damage in the foetus.

Other workers, however, state that there is no relationship between the diet of the mother and the prothrombin level of the newborn [159, 208, 844].

Javert and Stander [846] believe that a deficiency of not only vitamin K but also of vitamin C is necessary for the development of a hæmorrhagic diathesis in the newborn, and that deficiency of only one of them may fail to precipitate the condition.

The administration of barbiturates, such as sodium pentobarbital orally and sodium amyl bromoallyl barbiturate rectally, to produce analgesia in labour, is stated to result in a lowering of the prothrombin level of the blood of both the mother and the baby [889], although this has been denied [299]. Even small doses of barbiturates are said to affect the prothrombin level. This effect can be prevented by giving vitamin K to the mother in labour. Fitzgerald and Webster [889] record excessive bleeding in five newborn babies out of thirty, whose mothers had received barbiturate analgesia and no vitamin K. In a group of six hundred and forty-one whose mothers had received vitamin K there was no evidence of neonatal hæmorrhage.

Hæmorrhagic Disease of the Newborn. Clinical. The low prothrombin level of the blood in newborn infants satisfactorily explains the pathogenesis of hæmorrhage of the newborn, which is characterized clinically by hæmorrhage into the gastro-intestinal tract (melæna, hæmatemesis), bleeding from the cord (omphalorrhagia), nose, palate, the genito-urinary tract, vulva, and the suprarenals. The commonest first symptom is the passage of a large tarry stool, but the condition may be ushered in by hæmatemesis, bleeding from the umbilicus, pallor due to internal hæmorrhage, or the rapid enlargement of a cephalhæmatoma. The child may pass enormous quantities of blood in the stools and if the hæmorrhage is not arrested it may die on the fourth or fifth day. In the differential diagnosis of hæmorrhage of the newborn the following must be considered: birth trauma, infection and sepsis, congenital thrombopenia, constitutional fibrinopenia, hereditary hæmophilia and pseudo-hæmophilia. The incidence of hæmorrhage of the newborn is stated by various authorities to be from 1 in 200 to 1 in 500 births [861]. According to Kato [861] the incidence of hæmorrhagic disease of the newborn is probably in the region of 0.5 per cent. of births. Salmonsens's figures from the Oslo University Clinic are sixty-six cases, fourteen of whom died, out of nine thousand seven hundred and forty-eight infants born alive [122].

Although it is generally considered that hæmorrhagic disease of the newborn occurs between the second and sixth days after birth, it has been stated that in a third of one series studied it occurred on the first day [118]. Neonatal hæmorrhage *in utero* has also been reported by Javert [119], who suggests that uterine contractions increase the intracapillary pressure of

the foetus and tend to produce hæmorrhage, which becomes pathological when the clotting mechanism is disturbed.

Asphyxia Neonatorum. Edsall [202] examined the prothrombin level of twenty-seven infants most of whom had some neonatal accident. The levels ranged from five to seventy per cent. of the adult normal. Nine of eleven infants with asphyxia neonatorum or intracranial hæmorrhage had levels below thirty-five per cent. of normal. Although no absolute relation was observed between the prothrombin level and the tendency to hæmorrhage it was noted that infants with levels below fifteen per cent. tended to bleed severely. Infants with levels between fifteen and thirty per cent. sometimes bled and when they did hæmorrhage was severe. Those with levels above thirty per cent. showed a transitory melæna.

A marked prolongation of the prothrombin time and a tendency to spontaneous hæmorrhage was also noted by Ross and Malloy [250] in a group of babies suffering from asphyxia neonatorum. They suggest that anoxæmia may be a factor in the production of spontaneous hæmorrhage in the newborn; this is supported by observations on induced anoxæmia in newborn chicks, which results in a prolongation of the prothrombin time. In the light of these findings the prophylactic use of vitamin K might be of value in asphyxia neonatorum.

Intracranial Hæmorrhage. Because of its relative frequency and disastrous consequences intracranial hæmorrhage has always caused great concern among obstetricians, who have no doubt been wrongly accused in the past of unnecessary trauma at birth. Of the deaths of the newborn twenty-five to over fifty per cent. [342, 343] have been attributed to intracranial hæmorrhage, the incidence of which varies from hospital to hospital; it has been put at from one in twenty-five to one in one hundred and fifty deliveries [128]. Roberts [124] estimates that it occurs clinically in from one to two per cent. of all infants. This does not take into account the large army of mental and physical cripples who were so unfortunate as not to die as a result of their neonatal cerebral accident.

It is now believed by many that intracranial hæmorrhage is associated with hypoprothrombinæmia and that obstetric trauma may only play a secondary rôle [118, 115, 122, 124]. It is conceded that the trauma of birth may in many instances cause the appearance of small bleeding points in the intracranial cavity. However, even in normal births and in babies born by cæsarean section such bleeding points have been demonstrated, and death from cerebral hæmorrhage has been reported after cæsarean section [204]. With an abnormal clotting mechanism, such as will result from a temporary hypoprothrombinæmia, no clot will form and slow oozing will continue. With a normal clotting mechanism bleeding from small vessels would cease. This slow oozing from the bleeding points explains why the symptoms of intracranial hæmorrhage often only reveal themselves on the fourth to sixth day after birth. Thus out of sixteen cases of intracranial hæmorrhage at the Children's Hospital, Washington, D.C., the average date of onset was 6.8 days after birth [125]. Salmonsén's report that sixteen out of sixty-six infants with hæmorrhagic disease showed evidence of cerebral hæmorrhage suggests that hæmorrhagic disease in the newborn should include intracranial hæmorrhage [122].

Treatment of Hæmorrhage of the Newborn. Conclusive proof that vitamin K checks the hypoprothrombinæmia of the newborn and controls the hæmorrhagic disease associated with it has been advanced by Waddell and his associates [127], and by a number of other workers [112, 116, 124, 128-130]. Four hundred newborn infants were treated with vitamin K by Waddell and Lawson [113], with an incidence of hæmorrhage of only one per cent.; in two hundred and nineteen cases serving as controls and not receiving vitamin K the incidence of hæmorrhage was 10·4 per cent. Javert and Macri [252] gave a series of 556 newborn infants vitamin K at delivery and record that hæmorrhagic disease did not develop in a single



FIG. 207. Typical hæmorrhagia hypoprothrombinæmia neonatorum in a three-day-old infant. Hæmorrhage into the skin of shoulder and upper arm and from the umbilical cord (omphalorrhagia) can be seen. The infant responded to treatment with Menaphthone (2-methyl-1:4-naphthoquinone).

instance, although according to statistics of their previous cases not receiving vitamin K at least five would be expected to have the disease.

Lehmann [341] has also produced some convincing figures. From a comparison of deaths from bleeding in 18,250 infants receiving a water-soluble vitamin K analogue prophylactically with 17,740 untreated cases he was able to show that the lives of 1·6 infants per 1,000 born could be saved. As there are 750,000 live births per annum in the British Isles, vitamin K prophylaxis would, therefore, be expected to save 1,200 infants' lives a year at relatively little cost.

For the treatment of hypoprothrombinæmia of the newborn the dose is 1 to 2 mg. of menaphthone repeated until the prothrombin level returns

to normal. Larger doses, *e.g.*, 1 to 5 mg., of the water-soluble vitamin K derivatives may be given.

The observations of Sanford and his colleagues [258] are at variance with those of all workers in this field. From a study of 1,698 newborn infants they state that they have been unable to demonstrate any correlation between the prothrombin level and the frequency of what they term



FIG. 208. Delayed hæmorrhagic disease of the newborn, with intracranial hæmorrhage superimposed on a previous interventricular hæmorrhage. The infant, who shows marked opisthotonus, also had severe diarrhoea and oral hæmorrhage. Subsequent therapy with vitamin K and a blood transfusion failed to save the infant's life. Autopsy revealed petechial hæmorrhage in the brain and massive hæmorrhage into the ventricles. The diarrhoea undoubtedly contributed to the hæmorrhagic state and depressed the prothrombin level so low that a poorly formed clot at the site of a previous hæmorrhage was dislodged and further hæmorrhage resulted. It is probable that hæmorrhage into the ventricles had continued since birth, and that when they were distended opisthotonus developed.

"hæmorrhagic manifestations." The latter occurred in 6.59 per cent. of 711 newborn infants given vitamin K, and in 6.6 per cent. of 982 controls not receiving the vitamin. The mortality in the two groups was also practically the same (0.81 per cent. and 0.84 per cent.). This has led them to question the clinical value of administering vitamin K to the newborn. The explanation of these apparently anomalous results depends on the interpretation of "hæmorrhagic manifestation," and "hæmorrhage of the newborn." This work has been criticized by Waddell [254]. Certainly the

figures cannot compare with those of Lehmann [841], who reported on a total of 81,000 infants, or with those of Hellman and Shettles [255], of Johns Hopkins Hospital, Baltimore, who noted a mortality of 1.9 per cent. among 1,042 infants treated through the mother compared with a mortality of 8.9 per cent. amongst 1,206 untreated infants. Mathematical analysis of these figures shows that the odds against this being accidental are 194.7 to 1.

Prophylaxis of Hæmorrhage of the Newborn. A voluminous literature has developed on the prophylactic treatment of the newborn with vitamin K analogues [124, 126, 180, 181, 182, 189, 141, 142, 145, 175, 181, 182, 184, 185, 205, 207, 280, 246, 256, 257, 259, 261, 262]. Earlier workers gave doses that were too large. From a three-year study on 18,000 infants Lehmann [841] concludes that 0.5 to 1 mg. of sodium 2-methyl-1:4-naphthohydroquinone bisulphite orally or by mouth is an optimal dose. Other workers state that 1 mg. of menaphthone B.P.C. (menadione U.S.P.) by injection in oil or orally one to three times daily, or even in a single dose at birth, prevents hypoprothrombinæmia. Toohey [847] states that a single dose of 5 to 10 mg. of menaphthone raises the prothrombin time of the infant to adult levels. The official B.P. dose (B.P.C. 1942) is 5 mg. intramuscularly followed by 10 to 60 mg. acetomenaphthone orally, but this has never been revised in the light of recent work. Larger doses of some of the water-soluble analogues appear to be given, *e.g.*, tetra-sodium-2-methyl-1:4-naphthohydroquinone diphosphoric acid ester (5 to 10 mg.). The effective dose of sodium 2-methyl-1:4-naphthohydroquinone bisulphite is 1 to 4 mg., and of 4-amino-2-methyl-naphthol hydrochloride 1 to 2 mg.

It has been advocated that vitamin K be administered to mothers during the last few weeks of pregnancy or actually during labour to lower the incidence of neonatal hæmorrhage and possibly intracranial hæmorrhage. This was first done by Shettles and his colleagues [129, 255] at the Johns Hopkins Hospital and later by Waddell [188]. They gave vitamin K concentrates daily for two to five weeks before delivery. It is now known that repeated dosing of the mother with vitamin K is unnecessary, and that hypoprothrombinæmia in the newborn can be prevented by giving the mother a single injection of a vitamin K analogue four to twelve hours before delivery [124, 184, 168, 181-188, 185, 256, 844, 847, 249]. The dose of menaphthone given has been from 2 to 20 mg. The official dose is 25 or 50 mg. of acetomenaphthone at the beginning of labour; much smaller doses seem satisfactory. Macpherson [124] showed that no better results were obtained by large doses. Another scheme is to give the mother 2 mg. of menaphthone as soon as labour starts and to continue to give it six hourly [188]. The daily administration of 1 to 2 mg. for a month as advocated by some workers seems quite unnecessary [184, 257, 260]. Toohey [847] states that 20 mg. of menaphthone given to the mother two hours before delivery and 5 mg. to the infant at birth raise the prothrombin level to adult figures. Snelling [844] found that 5 mg. given to the mother within four hours of birth was effective. The dose of tetra-sodium 2-methyl-1:4-naphthohydroquinone diphosphoric acid ester for the mother before delivery is 10 mg. parenterally [256, 261, 888]. The

oral administration of 20 mg. as recommended by Plum [262] is only advisable if it is given well before birth. Fiechter [849] states that a dose of 20 mg. given five to ten days before delivery is satisfactory. 4-Amino-2-methyl-1-naphthol has also been given parenterally to the mother in doses of 1 mg. According to Bohlender [185] it does not matter whether it is given to the mother five minutes or twenty-four hours before labour if given intravenously.

Macpherson [207] recommends the administration of vitamin K to the mother or the newborn in cases of maternal toxæmia, premature labour, difficult or instrumental delivery, failure to breast feed, development of cerebral symptoms in the infant, development of a hæmorrhagic diathesis and cases in which an operation is to be performed within a week of birth. To this list Plum and Dam [850] add maternal nephritis during the pregnancy. Parks and Sweet [351], while they grant that the administration of vitamin K to the mother raises the prothrombin level of the infant, state that they failed to observe any reduction in the incidence of neonatal hæmorrhage compared with controls whose mothers received no vitamin K. On the other hand, Beck and his co-workers [188], who studied a series of about the same size as Parks and Sweet, have produced figures showing that only 0.5 per cent. of a group of infants whose mothers received vitamin K showed any evidence of neonatal hæmorrhage, in contrast with an incidence of two per cent. in a control group. The incidence of melæna neonatorum was reduced from 0.7 to 0.1 per cent., but that of intracranial hæmorrhage was only reduced from 1.4 to 0.4 per cent.

Macpherson [207] has also compared the treatment of hæmorrhage of the newborn by injection of whole blood into the buttock, by blood transfusion and by the injection of vitamin K analogues. His results show that the administration of vitamin K analogues in doses of 2.5 mg. is superior to the other forms of treatment, as judged by a maintained increase in the plasma prothrombin and the rapid control of hæmorrhage. In some cases vitamin K analogues were effective after hæmotherapy had failed. This has been confirmed by the observations of Willumsen and his co-workers [247] and by Gellis and Lyon [248]. The latter found that a single injection of 20 c.c. of maternal blood had little or no effect in checking the decline of the prothrombin index during the neonatal period.

Scobbie [848], however, states that blood given intravenously is more effective in treating hæmorrhage of the newborn than vitamin K, and a writer of a *Lancet* annotation [852] gives the advice that if hæmorrhagic disease develops after giving vitamin K to the mother or the infant, the latter should be transfused with fresh blood. Reiss and Schönberger [383] express the view that healthy infants do not need vitamin K as their prothrombin level automatically adjusts itself, and that the administration of vitamin K should be reserved for the premature and those with birth injuries.

Summarizing, it is probably advisable to give menaphthone 5 mg. intramuscularly or a water-soluble analogue 5 to 10 mg. intramuscularly or intravenously to the mother just before or as soon as labour commences, repeated if the labour is prolonged. An additional injection of 1 mg. of menaphthone may be given to the infant as a further precaution, and if

there is any sign of neonatal bleeding further doses of 1 to 5 mg. may be administered and repeated as necessary. Some workers believe that if frank hæmorrhagic disease develops it should be treated by immediate transfusion with fresh blood. There is, unfortunately, no relationship between the level of prothrombin in the mother and newborn child, so that mothers cannot be pre-selected for prophylactic treatment with vitamin K [251]. Hence the suggestion that vitamin K be given routinely to the mother.

Erythroblastosis foetalis. Dam and his co-workers [185] observed lowered prothrombin levels in the blood of infants suffering from *erythroblastosis foetalis*, *icterus gravis neonatorum* and congenital anaemia of the newborn. The prothrombin level of two infants with the condition was raised to normal levels with vitamin K. Mayman [186] records the satisfactory treatment of one case and Macpherson [207] two cases of *icterus gravis neonatorum* with vitamin K. It is now known that these recoveries were independent of vitamin K therapy, which only corrected a concomitant hypoprothrombinæmia. *Erythroblastosis foetalis*, which has an incidence of 1 in 4,000 pregnancies [244], is due to intravascular hæmolysis in the infant's blood vessels as a result of a reaction between the Rh* antigen of the infant's cells and an anti-Rh agglutinin transferred through the placenta from the maternal blood. As far as is known *erythroblastosis foetalis* is not related to hypoprothrombinæmia or vitamin K deficiency. The only satisfactory treatment is the intravenous transfusion of Rh-negative blood. This survives for a long time in the blood of the infant, whereas Rh-positive blood is usually rapidly destroyed.

Gastro-Intestinal Diseases of Infancy and Childhood. Diseases characterized by chronic or subchronic gastro-intestinal lesions often develop hypoprothrombinæmia with or without bleeding. The literature contains reports on post-operative hæmorrhage in the pyloric stenosis of infants after Ramstedt's operation. Garrahan, Thomas and Largaia [862] noted a severe hypoprothrombinæmia in a case of pyloric stenosis. The child was given 10 mg. of vitamin K pre-operatively and relatively little hæmorrhage occurred during and after the operation. The same authors recommend the pre-operative administration of vitamin K in debilitated infants.

Cases of congenital obstruction of the alimentary tract show hypoprothrombinæmia, partly because no food, and hence exogenous vitamin K, is consumed and partly because there is no bacterial synthesis of the vitamin. Since surgery is imperative to save life, the hypoprothrombinæmia should be corrected with vitamin K before any operative procedure. Grossman [258] quotes a case of congenital jejunal obstruction in which hæmorrhage occurring after operation was controlled with vitamin K, and one in which a child with duodenal stenosis was operated upon without vitamin K therapy and died from hæmorrhage.

Cœliac disease in children, like sprue and idiopathic steatorrhœa in adults, is sometimes associated with hæmorrhagic manifestations. Fanconi [89] suggested that this was due to vitamin K deficiency caused by

* The red cells of most human beings contain an agglutino-gen also found in the red cells of the rhesus monkey, whence the designation Rh.

maladsorption of fats. Other authors have confirmed the presence of hypoprothrombinæmia in these conditions [95, 170].

Pseudohæmophilia Hepatica and Hereditary Pseudohæmophilia. Pseudohæmophilia hepatica is a rare hæmorrhagic syndrome accompanying acute destruction of liver tissue in infectious and toxic states such as syphilis, acute yellow atrophy, and poisoning by such drugs as arsenic, chloroform and the sulphonamides. The bleeding is due to a decreased production in the liver of prothrombin or fibrinogen or both, with a resultant prolongation of clotting time. Kugelmass [126, 187] describes the treatment of a case of infective jaundice with hæmorrhagic manifestations that responded favourably to the administration of vitamin K. Another case of hæmolytic anæmia, due to the toxic effects of sulphanilamide, also improved when given vitamin K in the same dosage.

Hereditary pseudohæmophilia is a syndrome appearing in both males and females characterized by a hæmorrhagic tendency, prolonged bleeding time, normal clotting time and a normal platelet count. It may be present at birth or latent for many years, the hæmorrhage developing as a result of an inherent qualitative defect in the blood platelets. A case of this condition with a low prothrombin level was satisfactorily treated with vitamin K by Kugelmass [126]. He is careful to point out, however, that since the hæmorrhage is primarily determined by defects in the platelets, vitamin K therapy is only indicated when there is a coincident decrease in the prothrombin level.

Retinal Hæmorrhage. There have appeared in the ophthalmological, obstetrical and pædiatric literature numerous theories explaining the ætiology of retinal hæmorrhage in the newborn. Maumenee, Hellmann and Shettles [168] attribute it to vitamin K deficiency. They have observed that newly born infants with retinal hæmorrhage show lower prothrombin levels than normal infants, and they believe that the incidence of the condition is reduced by administering vitamin K to the mother before labour. Two milligrams of 2-methyl-1 : 4-naphthoquinone were given by mouth daily for at least four days before delivery. They point out that the vitamin has very little effect if given during labour. By giving expectant mothers 2 mg. of 2-methyl-1 : 4-naphthoquinone daily for four days before the onset of labour the incidence of retinal hæmorrhage was reduced to four per cent. compared with twenty-five per cent. in controls [255].

Pray and his co-workers [259] also report a reduction in the incidence of retinal hæmorrhage in the infants of mothers treated with 10 to 20 mg. of 2-methyl-1 : 4-naphthoquinone during or before labour. They obtained the best results by treating the mother before the onset of labour.

This reduction in the incidence of retinal hæmorrhage in newborn infants by means of vitamin K therapy is significant because of the possible relationship between retinal and intracranial hæmorrhage.

Effect of Vitamin K on Thrombosis. Morton, Shearburn and Burger [278] have investigated the effect of vitamin K on thrombus formation. The post-partum incidence of thrombosis was 0.14 per cent. in a group of seven hundred pregnant women given vitamin K; the incidence in a group of 5,728 controls studied over a ten-year period, who had not

received vitamin K, was 0.48 per cent. In animal experiments the leg veins of two groups of dogs, one of which received vitamin K, were traumatized and sections later removed and examined microscopically. There was no significant difference in the incidence of thrombosis in the two groups. It would appear from this that the administration of vitamin K does not result in a decreased incidence of thrombophlebitis.

Hæmoptysis. Levy [821], Bauer [287] and Sheely [226] state that the intensity and duration of hæmoptysis in tuberculous patients—who often show low prothrombin levels—s considerably diminished after vitamin K therapy. On the other hand Kaplan [854], Harrell and Ray [855], Farber and Miller [820] report that vitamin K is of no value in the control of hæmoptysis.

Abortion. Moore and his colleagues [858] observed that does fed a diet deficient in vitamin K to produce hypoprothrombinæmia and then mated invariably aborted from the tenth to the fourteenth day, and at autopsy retroplacental hæmorrhages were seen. They suggest that because hæmorrhage occurred at this site the placenta is unduly susceptible to vitamin K deficiency since the blood prothrombin level did not fall to critical hæmorrhagic levels. Javert and Stander [846] believe that a deficiency of vitamins C and K is a factor in certain cases of threatened and spontaneous abortion. They observed that in seventy-nine patients with threatened, spontaneous or habitual abortion vitamin C deficiency was found in sixty-nine per cent. and hypoprothrombinæmia in seventy-two per cent. The patients usually complained of skin ecchymoses, epistaxis, bleeding gums, and vaginal bleeding. A clinical study of thirty-three patients with threatened or habitual abortion showed that after treatment with vitamins C and K, progesterone, minerals and vitamin E the incidence of abortion was twenty and seven per cent. in the two groups, while in controls the abortion rate was one hundred per cent. in two groups of forty-six. As so many preparations were given it is difficult to evaluate the precise rôle of vitamin K in diminishing the incidence of abortion.

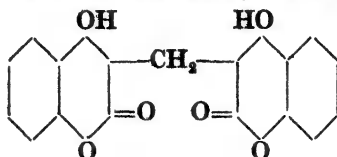
King [885] studied a hundred cases of abortion, and observed that in cases of inevitable and complete abortion the prothrombin level was lower than in pregnant and non-pregnant controls. In cases of threatened abortion the prothrombin level was within normal limits.

Drug Hypoprothrombinæmia. Certain drugs, such as organic arsenicals, sulphonamides, aspirin and salicylates are known to produce hypoprothrombinæmia (p. 810). It has been recorded that vitamin K therapy can raise a lowered prothrombin level when this is due to sulphonamide [306], aspirin and salicylate therapy [819]. Shapiro [819] has shown that approximately 1 mg. of menaphthone will counteract the prothrombinopenic activity of 1 gram of acetylsalicylic acid (aspirin) in patients receiving prolonged therapy with this drug. Hypoprothrombinæmia has been recorded in syphilitic patients given massive doses of organic arsenicals [812], but there is no record of this being corrected with vitamin K. Rawls [260] states that vitamin K lessens the toxic effects of cincophen by increasing liver function. The prothrombin level of the patients was raised by giving vitamin K, but there is no evidence that this

has any effect on liver function. It is generally recognized that vitamin K is ineffective if there is any hepatic damage (p. 824).

COMPOUNDS PRODUCING HYPOPROTHROMBINÆMIA

Dicumarol. Dicumarol, or 8,8'-methylene-bis-(4-hydroxycoumarin),



Dicumarol.

is a compound present in spoiled sweet clover which produces the condition known as the hæmorrhagic disease of cattle [10] by diminishing the prothrombin level [208]. It was first isolated from sweet clover and eventually synthesized in 1941 by Link and his associates [209] of the University of Wisconsin. Since then a considerable literature on the pharmacology and clinical applications of this compound has appeared.

Mode of Action. The oral administration of dicumarol to animals or human beings results in a reduction of the prothrombin level of the blood, as shown by a prolongation of the prothrombin time [210, 211]. There is a latent period of some twenty-four to seventy-two hours before the prothrombin time is affected. It has been suggested that dicumarol inhibits the formation of prothrombin in the liver and that the prothrombin in the blood is reduced before the prothrombin time is prolonged [278]. Quick [358] believes that it acts by diminishing or inactivating the B component of the prothrombin complex (p. 805), probably by inhibiting an enzyme mechanism which produces prothrombin. The action on prothrombin may not be a direct one; it may inhibit the activity of vitamin K. Dicumarol also prolongs clotting time, clot retraction time and red blood cell sedimentation rate; the bleeding time is not appreciably affected. Dicumarol decreases the adhesiveness of platelets, which play an important part in blood coagulation [368], and in large doses lowers the plasma fibrinogen concentration [377]. Experimental work on animals has shown that if the prothrombin time is prolonged by dicumarol, intra-vascular thrombosis is inhibited [279]. Dicumarol has been used clinically for the prevention and treatment of post-operative thrombosis and embolism, although it does not dissolve blood clot already formed. Davidson and MacDonald [280] have shown that dicumarol is only effective when it has reduced the prothrombin to about thirty per cent. of normal. Dicumarol does not appear to have any toxic effect on the liver apart from inactivating prothrombin.

Pharmacology. The principal toxic effects produced by excessive dosage are pulmonary oedema [281] and bleeding, which may occur from the nose, stomach, lung, bowel, kidney, bladder, skin or beneath the conjunctiva. The hæmorrhage is both gross and microscopic. In addition there is acute glomerular swelling, a toxic lymphoid tissue reaction [282], rapid respiration, general vasodilatation, irregular heart beat, congestion

of the liver, and after death rapid rigor mortis [283]. In the early studies on dicumarol it was considered that vitamin K was not a physiological antagonist and could not inhibit the excessive hæmorrhagic tendency produced by dicumarol [280]. It is now known that the doses of vitamin K given were too small. If given in adequate dosage, *e.g.*, 450 mg. to 1 gm., vitamin K analogues (vitamin K₁ oxide, menaphthone bisulphite) can inhibit the action of dicumarol [284], although there is a long latent period of about five days before the hypoprothrombinæmia is corrected. The protective action of vitamin K against the hypoprothrombinæmia produced by dicumarol is lessened if there is liver damage, *e.g.*, by tumours [874].

Dicumarol is effective orally, parenterally and rectally. A single dose will prolong the prothrombin and coagulation times for two to three weeks. Its rectal absorption, however, is irregular and the effect given by this route is uncertain [285]. It is more effective in animals in which pyrexia has been induced, and care should therefore be exercised when it is used clinically in febrile patients.

Dicumarol produces no changes in the white cell count, percentage hæmoglobin, blood sugar, icterus index, liver function tests, non-protein nitrogen, or urine [869].

Clinical Uses and Indications. Following the observation that dicumarol considerably reduces the incidence and degree of thrombus formation after the intravenous injection of sclerosing agents (*e.g.*, ethanolamine oleate) [279], the drug has been given to a number of patients for the treatment and prevention of thrombosis. Clinical evidence shows that carefully controlled and individual administration of dicumarol is effective in the prevention of post-operative venous thrombosis and pulmonary embolism [285-287], and in the treatment of venous thrombosis, thrombophlebitis [288-292] and the spread of thrombosis after embolism. These include cavernous sinus thrombosis, puerperal thrombosis, the thrombosis of arteriosclerosis (in cerebral and coronary vessels) and thromboangiitis obliterans, and retinal thrombosis. Scott and Lissimore [368] have recently treated a case of mesenteric thrombosis with it. Barker, Allen and Waugh [286] state that out of ninety-one surgical patients with post-operative pulmonary embolism or infarction treated with dicumarol, only two had a subsequent attack, although forty were expected to. Seventeen patients treated with the drug were expected to have a fatal pulmonary embolism, but none did. In a thousand surgical cases receiving dicumarol recorded by Barker and his colleagues [387] at the Mayo Clinic, the incidence of post-operative venous thrombosis and non-fatal pulmonary embolism was 1.1 per cent. and fatal pulmonary embolism 0.6 per cent.; the figures in the control subjects not given dicumarol were 48.8 per cent. and 18.8 per cent. In the same series the incidence of post-operative thrombophlebitis in the dicumarol treated group was 2.9 per cent., whereas in the control cases it was 10.6. Brambel and Loker [867] used dicumarol in post-traumatic arteriosclerosis and gangrene due to diabetes and frostbite. They concluded that it prevented the extension of established gangrene and prevented it developing in traumatic cases.

The advantages of dicumarol over heparin as an anticoagulant are that

it is relatively cheap, it can be given by mouth or intravenously, and its effect is prolonged. The cost of heparin is prohibitive and it must be given intravenously over a long period. However, dicumarol cannot be regarded as completely safe as many cases of severe and alarming bleeding and hæmorrhagic purpura have been recorded after its use [298]. The increased prothrombin time following its use persists for several days after its withdrawal. Crawford and Nassim [864] describe a case of retinal thrombosis in which there was a delayed response to dicumarol followed by a severe reaction to the drug. The reactions, which included hæmatemesis, hæmaturia, albuminuria and a prolonged increased prothrombin time after the drug was withdrawn, necessitated blood transfusion. Wasserman and Stats [294] record such well-marked individual variations in the response to dicumarol that they are unable to fix a standard dose. Douthwaite [865] and Evans [866] describe cases resistant to dicumarol, and Evans [866] also records a death from hæmorrhage. This was in a patient who had had a sympathectomy for a thrombosis of the leg. Bleeding into the wound, abdominal wall and retro-peritoneal tissues occurred after giving dicumarol and six blood transfusions did not stop it. Shlevin and Lederer [869] also record a case of fatal uncontrollable hæmorrhage after administering dicumarol; at autopsy there was hæmorrhage into the brain, kidneys, bladder, stomach and retroperitoneal tissues. Zucker [288] states that he avoids such accidents by plotting the prothrombin time daily and giving the daily dose of dicumarol only if it is less than the time recorded the day before. Barker and his colleagues [887] at the Mayo Clinic believe that the risk of bleeding after giving dicumarol has been exaggerated. They state that in 1,000 cases on the drug, only 8.9 per cent. showed evidence of minor hæmorrhage and 2.5 per cent. major hæmorrhage. Major bleeding was controlled by an intravenous injection of 60 mg. of menaphthone (menadione) bisulphite. They state that if the proper contra-indications are observed and the dosage carefully regulated, the danger of severe bleeding is slight.

There are definite contra-indications to the use of dicumarol. Absolute contra-indications are: renal insufficiency; purpura; hæmorrhagic diathesis; prothrombin deficiency, *e.g.*, in cases of jaundice, hepatic disease and malnutrition; blood dyscrasia with tendency to bleed; and subacute bacterial endocarditis. Relative contra-indications are: ulcerative lesions, open wounds, bleeding surfaces; possibility of operation within two weeks; operations on the brain or spinal cord; and vomiting due to gastric or intestinal drainage [286, 387]. Dicumarol should not be used as a routine without control after operation because of the danger of hæmorrhage.

Hypoprothrombinæmia can be induced in suckling animals if the mother is given dicumarol, and can be corrected by giving vitamin K to the mother [876]. Care should therefore be taken in giving dicumarol to nursing mothers for puerperal thrombosis.

Dicumarol Therapy [286, 294, 387]. Dicumarol should never be used unless daily and consistently comparable prothrombin time tests are done, as it is impossible to predict the dose for any one patient. The Quick prothrombin time is recommended [387]. Dicumarol is usually given in

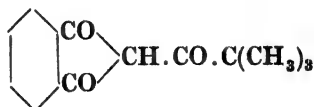
capsules of 100 mg. In cases of embolism or phlebitis 800 mg. are given to a patient weighing 100 lbs. as soon as the diagnosis is made, a dose of 200 mg. on the second day, and on each succeeding day that the prothrombin time is less than thirty-five seconds. If the prothrombin time is greater than this on any day, the drug is omitted. In cases that respond rapidly to dicumarol it may be necessary to give only 100 mg. and in resistant cases 800 mg. It should be remembered that effective prothrombin levels may not be reached until twenty-four to forty-eight hours, and even longer, after giving dicumarol. When given for prophylactic purposes after operation, treatment is started on the third post-operative day. The prothrombin time is kept at about thirty-five seconds (Quick) several days or a week after the patient has become ambulatory. Barker, Allen and Waugh [286] suggest sufficient dicumarol to keep the prothrombin time (Quick) between thirty-five and sixty seconds. After stopping treatment it takes two to ten days for the prothrombin time to return to normal. It may take longer than this in cases of liver or kidney damage or in patients with a hæmorrhagic tendency. The prothrombin time may be kept elevated for three months by a total dose of 10 grams of dicumarol without harm to the patient [296].

To get an immediate anti-coagulant effect dicumarol has been combined with heparin, the latter being discontinued when the prothrombin time reaches thirty-five seconds [296].

The use of dicumarol is not without risk as severe and even fatal hæmorrhage may occur for no apparent reason. Liver function tests should be carried out before treatment as liver damage renders both animals and human subjects more sensitive to the action of dicumarol [295]. Facilities for blood transfusions should always be available.

Hæmorrhage is treated by a blood transfusion of 500 c.c. of fresh (not bank) citrated blood, repeated several times if necessary, as there is a tendency for the prothrombin time to increase again after several hours. Large doses of a soluble vitamin K analogue, e.g., from 60 mg. to 1 gm., preferably intravenously, counteract the action of dicumarol.

Indandione Derivatives. Kabat, Stohlman and Smith [297] have shown that a number of indandione derivatives induce hypoprothrombinæmia. The compound 2-pivalyl-1:3-indandione



is stated to be as effective as dicumarol.

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CHAPTER XI

VITAMIN P

HISTORY

THE existence of vitamin P was postulated in 1936 by Szent-Györgyi [1] and his co-workers, who claimed that extracts of Hungarian red pepper and lemon juice contained a substance which was more effective in the clinical treatment of increased vascular permeability than vitamin C. Later it was reported that fractionation of these extracts yielded an active preparation consisting of a flavone or flavone glucoside, which was called *citrin* or vitamin P [2]. This was stated to be effective in the treatment of increased capillary permeability in three patients suffering from vascular purpura, but it had little effect on four patients with thrombopenic purpura. A moderate effect was observed on the capillary fragility in seven patients suffering from infectious disease, myxoedema and diabetes.

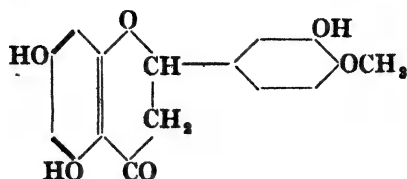
Szent-Györgyi [2] also stated that citrin decreased the number of hæmorrhages in scorbutic guinea-pigs and prolonged the survival period from 28.5 days for the negative control group to forty-four days for the animals given 1 mg. of citrin daily [8]. It was suggested that the full clinical syndrome of scurvy in the guinea-pig was produced by a combined deficiency of vitamins C and P.

Efforts to repeat the observations of Szent-Györgyi and his co-workers in experimental animals have yielded conflicting results. Thus Zilva [5], Moll [6], Hiramatsu [7], Detrick [8], Bensath and Das [9] and McHenry and Perry [88] were unable to confirm them. Zilva administered a vitamin P preparation (0.66 mg. hesperidin and 0.33 mg. eriodictyol daily) to guinea-pigs on a scorbutic diet, but he failed to observe any delay in the onset of scurvy or in the time taken for the animals to succumb. He claims that the administration of a daily dose of 0.1 to 0.2 mg. vitamin C to such animals produced a condition resembling that obtained by Szent-Györgyi and his colleagues by administering a daily dose of vitamin P. Szent-Györgyi [10] has since reported his failure to repeat his original experiments upon which the existence of vitamin P was based. The more recent work of Zacho [11], Bacharach [41, 50, 64] and Bourne [48] shows that vitamin P does affect capillary fragility in the experimental animal.

CHEMISTRY

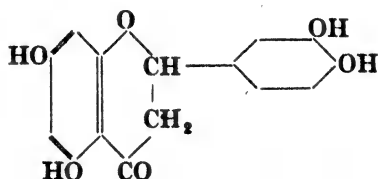
The chemistry of vitamin P was investigated by Szent-Györgyi [4], who considers that it consists of mixed crystals of two related flavone dyes, the glycoside hesperidin and the glycoside of eriodictyol. According to him the former constitutes the major part of citrin; the latter is responsible for the chemical reactivity and yellow colour. Hesperidin is

the glucose rhamnose glycoside of 5 : 7 : 8'-trihydroxy-4'-methoxyflavanol according to Szent-Györgyi :—



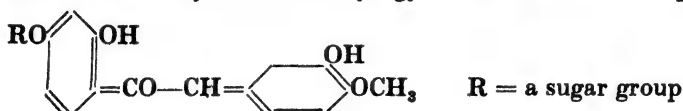
Mager [58], however, states that it is the *l*-rhamnose glycoside. According to Scarborough [65], hesperidin is not vitamin P, as there is an active material in rose hips at least two and a half times more active.

Szent-Györgyi believes that eriodictyol glycoside is the glycoside of 5 : 7 : 8' : 4'-tetrahydroxy-flavanol :



This work lacks confirmation and the chemical identity of the vitamin must still be considered doubtful.

Wawra and Webb [46] claim to have discovered a new oxidation-reduction enzyme containing vitamin P as the prosthetic group. They believe that the eriodictyol of Szent-Györgyi is the chalcone of hesperidin,



and that it is capable of being reversibly transformed by reduction and ring closure to the flavanone glycoside. Preliminary experiments showed that the chalcone decreases capillary fragility and prevents hæmorrhage.

The flavones, which are naturally occurring yellow plant pigments, are phenyl derivatives of 1 : 4-pyrone. They occur in plants, fruits and flowers as glycosides, in which glucose or rhamnose form the carbohydrate part of the molecule. They are water soluble.

Goldfarb, Bueding and Karp [52] have described a method for extracting citrin from lemon peel in the cold.

ESTIMATION AND UNITS OF VITAMIN P

A method of estimating vitamin P based on the measurement of capillary resistance has been developed by Bacharach, Coates and Middleton [41]. They apply a suction cup with a diameter of 12 mm. to the greased and shaven area on the back of a guinea-pig, the pressure being gradually reduced by 5 mm. stages, and maintained at each stage for three to five seconds. The pressure is noted at which petechiæ are first seen when the area under suction is viewed through the cup. This is

called the critical petechial pressure. If this is plotted against the logarithm of the dose of vitamin P administered to the animals, a straight line graph is obtained. A similar technique is used by Bourne [48], who uses a suction cup 20 mm. in diameter, the pressure being rapidly reduced to the desired level for ten seconds and then released. The capillary resistance is then taken as the negative pressure required to produce so many petechiae that the area under test becomes uniformly reddish-purple in colour. Animals on a scorbutogenic diet show a steady and significant fall in capillary resistance not corrected by vitamin C. It is abolished by hesperidin and the citrus glycosides.

Vitamin P Content of Fruits and Vegetables
(Bacharach and Coates)

Food.	Description.	Part tested.	Vitamin P content in "units" per 100 g.
Apple . . .	Bramley's seedling	fruit	60
Beetroot . . .	—	root	15
Blackberry . . .	—	fruit	60
Blackcurrant . . .	purée = 65 per cent.	raw fruit	500
" . . .	fresh	fruit	75*
Cabbage . . .	spring (April)	leaves	60
" . . .	summer (October)	"	100
Carrot . . .	April crop	root	10
" . . .	August crop	"	40
Cauliflower . . .	—	flower	40
Cherry . . .	black	fruit	60
" . . .	white	"	50
Dandelion . . .	—	leaves	80
Dock . . .	—	"	20
Lemon . . .	fruit	peel	500
" . . .	"	juice	450
Lentil . . .	—	seed	0
Lettuce . . .	round (May)	leaves	80
" . . .	" (September)	"	100
Maple pea . . .	dried	seed	10
Orange . . .	fruit	juice and peel	400
Parsley . . .	—	leaves	130
Parsnip . . .	—	root	40
Pea . . .	dried	seed	40
" . . .	germinated	"	80
Pea, maple . . .	dried	"	10
" . . .	germinated	"	10
Plum . . .	Victoria	fruit	50
Potato . . .	old (April)	tuber	25
" . . .	new (July)	"	40
Rhubarb . . .	—	stalks	20
Rose hips . . .	—	fruit	240-380
" . . .	" syrup	"	350
Rowan . . .	—	"	300
Spinach . . .	—	leaves	180
Swede . . .	—	root	20
Tomato . . .	—	fruit	60-70
Turnip . . .	—	root	20-30
Walnut . . .	—	kernel	100
Watercress . . .	April crop	shoots	10
" . . .	October crop	—	70

* Bacharach attributes this anomalous result to the guinea-pigs used for the estimation showing "little inclination to co-operate."

Bacharach and Coates [50] express the activity of a vitamin P preparation under test in terms of a standard water-soluble calcium-containing glycosidic complex derived from citrus peel, which is similar to Szent-Györgyi's citrin. One "provisional unit" (P.U.) is defined as the activity of 1 mg. of this preparation. Recrystallized hesperidin had an activity of 100 P.U. per gram. Bacharach and Coates [64] have now prepared a vitamin P standard of potency 160,000 units per 100 gram. Black-currant concentrates of 1,060,000 units per 100 gram have been obtained.

Distribution of Vitamin P in Foodstuffs

Generally speaking fruits appear to be the richest source of vitamin P, followed by green leaves. There is very little vitamin P in roots and seeds, although there is an increase in the latter on germination. According to Bacharach [51] there is little vitamin P lost in the processing of commercial fruit concentrates and syrups, but some occurs on storage. The figures on p. 859 giving the vitamin P content of fruits and vegetables in the unit devised by Bacharach and Coates are taken from a table published by them [50].

The potency of hesperidin is 9,500 units of Bacharach and Coates per 100 grams.

Scarborough [58], on the basis of determinations of alterations of capillary resistance in man, states that grapes, grape juice and prunes are good sources of vitamin P, and that grape fruit juice and pulp and dried apricots are moderately good sources.

Although vitamins P and C are associated in fruits and vegetables there is no correlation between vitamin C content and vitamin P activity in the same food [64].

PHYSIOLOGY AND PHARMACOLOGY OF VITAMIN P

The work of Zacho [11], Bacharach [41, 50, 64] and Bourne [48], suggests that vitamin P or citrin plays a part in the control of capillary permeability in the experimental animal. The low capillary fragility of scorbutic guinea-pigs rises after giving vitamin C, but still keeps at a sub-normal level. When citrin is added to the diet, however, the capillary fragility reaches normal levels. The intestinal hæmorrhages of scorbutic animals also clear up when vitamin P is added to the diet. Rusznyak and Benko [81] found that lowered capillary resistance in rats could be raised to normal levels in ten to fourteen days by subcutaneous injections of 8 to 4 mg. of citrin. Todhunter [15] and his co-workers report that scorbutic guinea-pigs receiving supplements of lemon juice show fewer hæmorrhages than do animals receiving equivalent amounts of pure synthetic vitamin C. They observed that on a scorbutic diet reinforced by synthetic vitamin C the capillary fragility decreased considerably, but rose to normal levels or above after the administration of vitamin P or citrin.

Using a new technique for demonstrating capillary fragility Majovski and others [49] have shown that crude hesperidin from oranges and a water-soluble extract of lemon peel afford protection to mice against hæmorrhage

for two to four hours. The technique they use is to withdraw air suddenly from a jar in which mice are placed; this produces hæmorrhage into the lungs. The method is criticised by Kilbrick and Goldfarb [67], who have been unable to reproduce these results.

Schmid and Saubermann [57] claim that the administration of citrin in doses of 75 to 150 mg. decreases the intraocular pressure in rabbits with hydrophthalmus, which they, therefore, consider to be associated with a defect in capillary permeability.

Levcowich and Batchelder [47] noted in a group of women on scorbutic diets given graded amounts of synthetic vitamin C that the capillary fragility was not affected by administering vitamin C alone. They supposed that vitamin P was lacking in the diet employed.

Hartzell and Stone [54] have shown that vitamin P, unlike vitamin C, plays no part in the healing of wounds.

Vitamin P is absorbed when given orally, and it is active when administered parenterally. It is excreted in the urine [87]. Apparently vitamin P is not stored to any appreciable extent in the tissues [86]. After the administration of test doses of 50 to 100 mg. of citrin or eriodictyol intravenously about fifty per cent. is excreted in the urine [87]. Saturation of the tissues is reached in normal persons in from two to six days, but in disease a much longer period is required. In vascular purpura saturation cannot be attained [87].

There is some evidence that vitamin P, like vitamin C, can inactivate the toxin of diphtheria [88]. It is not as effective as vitamin C in protecting guinea-pigs against a full lethal dose of diphtheria toxin. Unlike vitamin C it has no bacterio-toxic action, although it appears to reinforce the action of this vitamin.

In health vitamin P has no effect on the serum albumin/globulin ratio, but in disease the serum albumin is decreased from 5.7 to 3.1 mg. per cent., and the serum globulin increased from 3.2 to 5.1 per cent. In this respect its effect is the opposite to that of vitamin C.

Citrin causes a slight fall in blood pressure when administered intravenously; a dose of 100 mg. causes a fall of 10 to 15 mm. after about three to four seconds [89]. This fall is apparently due to vasodilatation. The vitamin has very little effect on hæmopoiesis in normal animals, although in the scorbutic guinea-pig there is a twenty to thirty per cent. increase in the reticulocytes within a few days [40].

HUMAN VITAMIN P DEFICIENCY

A number of clinical studies suggest that vitamin P is an essential factor in human nutrition. The observations of Scarborough [12-14, 65] appear to establish with some certainty the existence of a dietary factor essential for the maintenance of capillary resistance in human beings. He observed an increased capillary fragility in a number of patients suffering from multiple vitamin deficiencies of varying degrees of severity. In two patients with scurvy the daily administration of 3 grams of hesperidin and a rose hip preparation containing 550 units (Bacharach and Coates) produced a significant rise in capillary resistance [65]. Previous treatment

with vitamin C had produced a fall. The capillary resistance was determined both by Borbely's negative pressure method, in which negative pressure is applied to the skin by means of an evacuated glass cup and increased until petechiæ form, and also by the positive pressure method. In the latter case the number of ruptured capillaries is counted over a given area after obstructing the venous return to the arm for a certain time. Scarborough observed that orange and lemon juice and extracts made from them produced an increase in the resistance of capillary walls of these patients, even when vitamin C had failed to produce any effect. The precise chemical nature of the substance or substances present in the extracts used by Scarborough was not established.

In a later study Scarborough [14] concluded that at least two forms of subcutaneous bleeding may develop as the result of nutritional deficiency in man. One form is due to vitamin C deficiency and is characterized by the large subcutaneous hæmorrhages (ecchymoses) seen in the scorbutic state and involving considerable areas of subcutaneous tissue and muscle. Gingival bleeding is also common. Vitamin P does not control these hæmorrhages, which are only arrested by large doses (500 mg.) of vitamin C. Scarborough also reported that vitamin P has no effect on the other important manifestations of the scorbutic state—tissue hydration, gingival hæmorrhage, anæmia, knee flexion, and general clinical condition. But apparently vitamin P can increase the capillary resistance of scorbutic subjects either before or after treatment with vitamin C. The capillary resistance of such subjects is not controlled by vitamins A, B₁, C or D. According to Scarborough [65] the minimum daily requirement of vitamin P to maintain capillary resistance at a high level is under 550 "provisional units" (p. 860) and probably under 300. Three grams of hesperidin would supply the latter quantity.

Cameron and Mills [55] gave vitamin P but not vitamin C to a patient with classic scurvy. The hæmorrhagic features promptly disappeared although the other symptoms of the disease were unaffected until vitamin C was given.

Scarborough believes that a deficiency of vitamin P may exist in man even after dosage with large quantities of vitamin C. The clinical manifestations of vitamin P deficiency that he describes include pains in the legs on exertion, pain across the shoulders, weakness, lassitude and fatigue. It is invariably associated with a much decreased capillary resistance and may be characterized by the development of spontaneous petechial hæmorrhages, especially in areas exposed to pressure (*e.g.*, of tight clothes). It has not been found to be accompanied by any hæmatological abnormality, and it responds to treatment with vitamin P (50 mg. doses). The hæmorrhages developing as a result of vitamin P deficiency are always small (petechiæ) and take place into the skin. They are often circumpilar. Hæmorrhage is more severe in parts exposed to the pressure of clothing and in the legs because of the higher venous pressure in the latter. Scarborough considers that some forms of purpura may have a nutritional basis, although in a later paper he states that vitamin P is ineffective in its treatment [56]. He has recently described an experimentally induced clinical syndrome in two human subjects attributable to

a deficiency of vitamin P [82]. The major features of this syndrome are petechial bleeding, low capillary resistance, and a slightly prolonged bleeding time. A case of orthostatic purpura, claimed to be due to vitamin P deficiency, has also been described [48].

CLINICAL USES OF VITAMIN P

Various vitamin P preparations have been used clinically, *e.g.*, citrin in doses of 20 to 60 mg. daily, either orally or parenterally; hesperidin 0.25 gm. intravenously to 1 gm. a day orally; and calcium eriodictyol 25 mg. In some cases preparations of lemon of unknown potency have been given.

Capillary tonus may be decreased as a result of trauma, pressure, avitaminosis, bacterial invasion, chemical injury or lymphatic infiltration. Kugelmass [16] claims that he has been successful in treating several of these conditions with vitamin P in doses of 150 mg. a day by mouth. He describes four cases of purpura of nutritional, allergic, infectious and traumatic origin, all of which were treated successfully with vitamin P. A dystrophic premature child of five months failed to thrive and showed purpuric spots on the abdomen. Although these began to clear under treatment with blood transfusions and lactic acid milk, they cleared more rapidly when vitamin P was given. A girl of ten years with allergic purpura responded slightly to calcium gluconate, rapidly to vitamin P, relapsed when this was withdrawn and improved once more when it was given again. Another girl of fifteen had recurrent cutaneous hæmorrhages associated with fever, abdominal pain and diarrhœa. Her condition improved after the elimination of offending allergens, but the purpuric manifestations remained. The number of petechiæ, however, was considerably reduced when vitamin P was administered. Another case of purpura following scarlet fever showed improvement under vitamin P therapy. Cases of traumatic petechiæ in the conjunctiva resulting from attacks of whooping cough and epilepsy, and orthostatic purpura from the pressure of plaster casts, failed to show any improvement when given vitamin P.

The œdema that characterizes most of the toxæmias of late pregnancy has been ascribed, in part at least, to an increased capillary permeability. Shute [85] chose a group of twenty pregnant women with gross œdema of the extremities in association with late toxæmia, and having excluded other causes of the œdema, the patients were given 60 to 120 mg. of vitamin P daily; four received 900 mg. a day. None of them had signs of vitamin P deficiency as described by Scarborough. However, the visible œdema and the patients' weight were unaffected in all cases.

The capillary resistance in a group of a hundred allergic children was studied by Rapaport and Klein [29]. Forty-nine had an abnormal capillary resistance and of these twelve were selected for treatment with vitamin P, which was given in doses of 100 mg. by mouth every day for six months. After this time the capillary resistance tests became normal in all the twelve children, although two did not respond to 100 mg. of the vitamin and required doses of 150 mg. a day. A vitamin C deficiency was excluded by giving vitamin C to all those children showing blood ascorbic

acid levels below normal. Rapaport and Klein believe that vitamin P plays an important rôle in the permeability mechanism of the capillary wall. Bell, Lazarus and Munro [68] were unable to show that the administration of vitamin P significantly increased the capillary resistance of apparently healthy persons.

Rudy, Beaser and Seligman [62] gave numerous vitamin P preparations, including lemon juice, lemon extract, hesperidin and calcium eriodictyol to diabetic patients with a definitely increased capillary fragility, but with no beneficial results. Negative results were also obtained with vitamin P given to patients with thrombopenic purpura and rheumatoid arthritis who showed decreased capillary resistance.

Jersild [17] describes the case of a woman suffering from Schönlein-Henoch purpura for eight years with symptoms involving the bowels, skin, joints, and urinary tract, and a lowered capillary resistance. Treatment with vitamin C (2,400 mg.) over a period of one week failed to effect the course of the disease, but intravenous injections of 50 mg. of vitamin P brought about a complete disappearance of all symptoms, even when the administration of vitamin C was stopped. When the injections of vitamin P were discontinued the capillary resistance fell and new petechiæ appeared, but this was reversed when more vitamin P was administered.

Further claims have been made for the treatment of five cases of purpura in Japan [25]; three Schönlein-Henoch, one purpura rheumatica, and one purpura simplex. In doses of 0.25 to 1.0 gram vitamin P was claimed to be clinically effective in raising a lowered capillary resistance, and to be potentiated by the simultaneous administration of vitamin C. The total amount of vitamin P given in each case was from 8.5 to 25.0 grams. Franke [26], however, who has used vitamin P, both alone and with vitamin C in cases of lowered capillary resistance, reports that it has no effect on the latter at all.

Miller [28] gives an account of a case of thrombocytopenic purpura developing in the convalescent stage in measles that had proved refractory after treatment with fruit juice and calcium gluconate. A vitamin P preparation was then given in doses of 0.25 gm. at two-hourly intervals. There was slight improvement after 8 gm. had been given, *i.e.*, in about twenty-four hours epistaxis and oozing from the gums cleared. After another 8 gm. had been given there was marked relief from hæmorrhage and fewer red cells in the urine. Then the dosage was altered to four-hourly intervals, making a total of 11.25 gm. in five days. After eight days the capillary resistance test was negative, no fresh petechiæ had appeared, and the urine was free from red cells.

Another flavone glucoside, rutin, structurally related to hesperidin, is stated to influence capillary resistance. Rutin is present in tobacco, garden rue, forsythia, elder flowers and violets. Griffith, Couch and Lindauer [66] gave rutin in doses of 20 mg. two or three times daily to eleven patients with hypertension, who showed a decreased capillary resistance. The patients were studied for a period of from twelve to sixteen weeks; capillary resistance was stated to become normal in eight patients within two months. Two subjects stopped taking the rutin and within six weeks the capillary resistance had fallen again.

Scarborough [82] records two cases diagnosed as vascular purpura (*purpura senilis*) that responded to treatment with a vitamin P preparation.

Vacek [84] has observed that in thrombopenic subjects the administration of vitamin P caused the disappearance of petechiæ, although it had no effect on the blood platelets. In two cases of hæmorrhagic purpura vitamin P was stated to control the disease and to enable the patients to weather the acute attacks. Vacek confirms the observation that vitamin P shortens blood-clotting time, and that it has no influence on the bleeding time or the thrombocyte count.

The decreased capillary resistance following the injection of arsphenamine was noted by Borbely [18], and Scarborough and Stewart [12] have shown that the decreased resistance occurring as a toxic manifestation during antisyphilitic treatment with arsenic and bismuth can be increased by giving vitamin P in doses of 1 gram daily. Horne and Scarborough [19] have also found that the toxic erythema and dermatitis that sometimes result from arsenic therapy in the treatment of syphilis are also associated with a low capillary resistance, which responds to treatment with vitamin P. Gorrie [20] reports a case of acute purpura hæmorrhagica developing after injections of neoarsphenamine, with profuse hæmaturia, retinal hæmorrhage, and necrotic angina. No vitamin C deficiency was present. Recovery rapidly occurred after the patient was treated with 1 gm. vitamin P orally for three days and a blood transfusion.

The rapid treatment of syphilis in a matter of days with massive doses of arsenicals has attracted considerable attention recently, but the toxic reactions have prevented its routine adoption. The most serious toxic manifestation is arsenical encephalopathy, which develops in one to two per cent. of patients [59]. The commonest clinical finding is capillary damage, which suggests that organic arsenicals cause an increase in capillary permeability. The investigations of Goldstein, Stolman and Goldfarb [60] suggest that the methyl chalcone of hesperidin may diminish the toxic effects of organic arsenicals such as mapharsen without inhibiting its treponemicidal effect, and that it may actually possess a mild treponemicidal activity itself. These workers administered 10 to 80 mg. of the methyl chalcone of hesperidin per kilogram of body weight daily for seven days to rabbits before treatment with toxic doses of mapharsen. In the treated group the survival rate was ninety per cent.; in the untreated group it was fifty-seven per cent. These preliminary observations suggest that the methyl chalcone of hesperidin might be of value clinically in diminishing the toxic effect of large doses of organic arsenicals.

Claims have been made that hæmorrhagic nephritis and hæmaturia respond to treatment with vitamin P. Thus Lajos [21] states that it was effective in doses of 25 to 800 mg. daily in the treatment of five cases of hæmorrhagic nephritis, one of intestinal hæmorrhage, one of gingival hæmorrhage, and one of endocarditis. Gimsing [22], on the other hand, gave vitamin P to seven patients with hæmorrhagic nephritis and obtained results that were no better than those obtained by rest in bed, careful nursing and dieting. In some cases vitamin P had an irritating effect on the kidney.

Raunert [28], who has used vitamin P in thirty cases of hæmaturia,

claims that it arrests hæmorrhage by raising the calcium level in the blood and by slightly lowering the blood pressure. She states that vitamin P in doses of 55 to 100 mg. by mouth every four hours effectively controls hæmaturia and raises the blood calcium level. The cases of hæmaturia were classified as being due to (1) non-specific inflammation, *e.g.*, nephritis, (2) renal hæmorrhage, (3) tumours, (4) operative interference such as cystoscopy and prostatectomy. In cases of nephritis doses of 100 mg. of vitamin P arrested the hæmorrhage after one or two doses. Hæmorrhage due to tumours ceased in three to seven hours after giving vitamin P, and that following operative intervention was arrested in twenty-four or at least forty-eight hours. Raunert describes a case of hæmorrhage from a papilloma of the bladder that resisted four months' treatment but stopped in a few hours after two injections of 55 mg. of vitamin P.

Two cases of ocular hæmorrhage satisfactorily treated with vitamin P are described by Mathewson [61]. In one case extensive retinal hæmorrhage, nasal and bladder hæmorrhage, probably caused by X-ray "spray" treatment, ceased almost immediately on giving vitamin P. No fresh retinal hæmorrhage occurred and those present were absorbed. In another case recurrent hæmorrhage that occurred into the anterior chamber of the eye after cataract extraction ceased after giving vitamin P.

Decker [24] has used vitamin P in cases of nasal and gingival hæmorrhage, and he believes that it is more effective when used with vitamin C. Blood studies showed that it lowered the bleeding time and increased the platelet count. No details of cases, or even their number are given, but he states that excellent results were obtained in the treatment of acute hæmorrhagic glomerulonephritis, chronic nephritis, and Werlhoff's disease. Kohl [44] describes the treatment of hæmorrhagic pachymeningitis with vitamin P. One to 3 c.c. of a 2.5 per cent. solution of citrin was given intravenously with the result that two patients died, seven were cured, apparently permanently, and two were cured temporarily, but relapsed later. Treatment in some cases was continued for seven to eight weeks—long enough for spontaneous improvement.

Vitamin P has been used for the treatment of psoriasis by Goldfarb [80], who divided forty-two patients with the disease into three groups. Group one, consisting of nine patients, were given crystalline citrin from lemon peel jelly corresponding to 267 mg. of eriodictin; four showed improvement and five no change. Improvement varied from lessening of the induration of the plaques to involution of the lesions as shown by central clearing. Twenty-two patients in the second group were treated with lemonade made from the whole lemon, corresponding to 800 mg. of eriodictin, and with 70 mg. of vitamin C a day for four months. Nineteen showed improvement and three no change. Ten patients in the third group were given vitamin C alone, but improvement was only noted in two cases, five becoming definitely worse and the remaining two showing no change.

At the moment it must be admitted that evidence for the clinical value of vitamin P in the absence of a deficiency of the vitamin is very scanty. From Scarborough's work it may be taken as established that an increased capillary fragility is frequently an indication of a deficiency of vitamin P.

A deficiency of the vitamin, however, is not the only circumstance in which a decreased capillary resistance is found, nor has it been established that any form of purpura is primarily due to an increased fragility of the capillary walls. Many of the clinical studies, particularly those on hæmorrhage and purpura, are open to criticism on the ground that they are conditions which may show improvement or spontaneous remission in the absence of any form of therapy. Also an insufficient number of cases have been taken for controlled studies to be made. Scarborough [27] points out that the presence of extravascular blood, either in the tissues or in the alimentary canal, markedly increases the capillary resistance and so makes petechial counts as a measure of the latter very inaccurate. Thus after the development of a purpuric eruption resulting in the extravasation of more than 4 c.c. of blood into the tissues, the capillary resistance will be high for two to four days and the individual may show a decreased tendency to the development of further purpuric spots during this period. Failure to recognize this means that entirely fallacious conclusions may be drawn from observations on the therapeutic value of vitamin P in purpura. Some of the conflicting results may also be due to differences of technique used by various workers. It is known for example that there is no correlation between the negative and positive pressure methods (p. 540) for measuring capillary fragility [63]. The negative pressure method, which has been employed by a number of workers, is stated by Rudy, Beaser and Seligman [62] to be unsatisfactory.

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